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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C07H 21/00 — A1

(11) International Publication Number: WO 97/27206

(43) International Publication Date: 31 July 1997 (31.07.97)

(21) International Application Number: PCT/US97/01236

(22) International Filing Date: 24 January 1997 (24.01.97)

(30) Priority Data:
08/592,255 26 January 1996 (26.01.96) US

(71) Applicant: CODON PHARMACEUTICALS, INC. [US/US]; 200 Perry Parkway, Gaithersburg, MD 20877 (US).

(72) Inventors: KHORLIN, Alexander, A.; 10634 Tuppence Court, Rockville, MD 20850 (US). WATANABE, Kyoichi, A.; 808 Gallop Hill Road #I, Gaithersburg, MD 20877 (US).

(74) Agent: KARTA, Glenn, E.; Codon Pharmaceuticals, Inc., 200 Perry Parkway, Gaithersburg, MD 20877 (US).

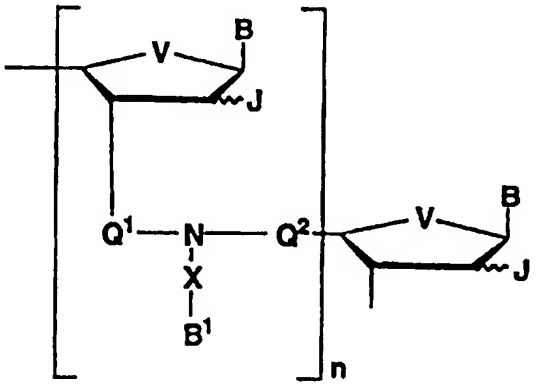
(81) Designated States: AL, AM, AU, BB, BG, BR, CA, CN, CZ, EE, FI, GE, HU, IL, IS, JP, KG, KP, KR, LK, LR, LT, LV, MD, MG, MK, MN, MX, NO, NZ, PL, RO, SG, SI, SK, TR, TT, UA, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: OLIGONUCLEOTIDE ANALOGS

(57) Abstract

Macromolecules are disclosed which contain the structure (1) wherein the substituents are defined in the specification. Also disclosed are compounds useful in the synthesis of the above compounds, as well as pharmaceutical compositions containing the above compounds as an active ingredient.



(1)

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OLIGONUCLEOTIDE ANALOGS

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates to a class of oligonucleotides, modified oligonucleotides, or oligonucleosides (*i.e.*, phosphate-free oligonucleotides) optionally modified at the ends and having in their structure at least one linker of a non-nucleosidic nature containing at least one (*aza*) nitrogen as an achiral site for attachment of a DNA-binding group or a DNA-interacting group or carrier or targeting ligand.

2. Description of Related Art

Oligonucleotides can be of value as therapeutic agents for the treatment of a wide variety of diseases. Classical therapeutics has generally focused on interactions with proteins, which either acting directly or through their enzymatic functions, contribute in major proportion to many disease states in animals and man. Compared to classical therapeutics, oligonucleotides offer the potential for a highly efficient specificity due to their capability for base pairing with complementary nucleic acid strands in a Watson-Crick or Hoogsteen manner. In that sense oligonucleotides provide a unique opportunity for gene therapy or the regulation of translation or transcription.

The function of a gene starts by transcription of its information to a messenger RNA (mRNA) which, by interaction with the ribosomal complex, directs the synthesis of a protein coded for by its sequence. The synthetic process is known as translation. Translation process requires the presence of various cofactors and building blocks, the amino acids, and their transfer RNAs (tRNA), all of which are present in normal cells.

Transcription initiation requires specific recognition of a promoter DNA sequence by the RNA-synthesizing enzyme, RNA polymerase. In many cases in procaryotic cells, probably in all cases in eucaryotic cells, this recognition is based on sequence-specific binding of protein transcription factor to the promoter. Other proteins which bind to the promoter, but whose binding prohibits action of RNA polymerase, are known as repressors.

Synthetic oligonucleotides could be used as "antisense" probes involved in binding to transcellular RNA in a sequence-specific fashion such as Watson-Crick base pairing interactions. Thus, synthetic DNA could suppress translation *in vivo*. It also may be possible to effect the genome by, for example, triple helix formation using oligonucleotides or other DNA recognizing agents.

Natural oligonucleotides, however, are relatively ineffective as therapeutic agents due to their poor permeability into the cell, and their rapid degradation by enzymes inside the cell. Therefore, relatively high concentrations of natural oligonucleotides are needed in order to achieve therapeutic effect.

In order to improve half life as well as membrane penetration, a large number of variations in polynucleotide backbones has been undertaken, although so far not with desired results. These variations include the use of methylphosphonates, monothiophosphates, dithiophosphates, phosphoramidates, phosphate esters, bridged phosphoramidates, bridged phosphorothioates, bridged methylenephosphonates, dephospho internucleotide analogues with siloxane bridges, carbonate bridges, carboxymethyl ester bridges, acetamide bridges, carbamate bridges, thioether, sulfoxy, sulfono bridges, various "plastic" DNAs, alpha-anomeric bridges, and borane derivatives.

For example, United States Patent 5,216,141 relates to DNA analogs containing sulfides, sulfoxides and sulfones as linking groups between subunits capable of forming bonds with natural oligonucleotides.

United States Patent 5,034,506 relates to polymeric compositions containing morpholino subunits linked together by achiral linkages. Each subunit is said to contain a purine or pyrimidine base pairing moiety.

United States Patents 5,405,938 and 5,166,315 relate to polymers containing an uncharged 5- or 6- membered cyclic backbone having selected bases attached to the backbone. The polymer is said to be able to bind in sequence specific manner to a target sequence of a duplex polynucleotide.

International applications WO 92/20702, 92/20703 and 94/25477 all relate to the so-called peptide nucleic acids (PNAs) which are said to bind complementary single stranded DNA and RNA strands more strongly than a corresponding DNA. The PNAs are said to comprise ligands linked to a peptide or polyamide backbone via an aza nitrogen.

United States Patent 5,378,825 is directed to oligonucleotide analogs in which the normal phosphorodiester inter-sugar linkages are replaced with four atom linking groups.

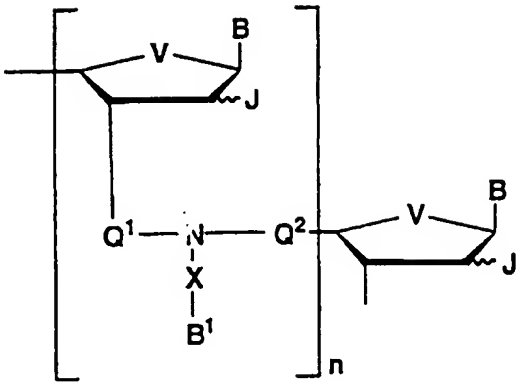
International application WO 95/14706 is directed to PNA-DNA-PNA chimeric macromolecules, wherein the PNA and DNA portions are connected by amide linkages, amine linkages or ester linkages.

Today it has been recognized that nuclease resistance, fidelity of binding, and efficiency of binding of antisense or antigen oligonucleotides to a target RNA strand or double-stranded DNA are of great importance. At the same time there has been a long-felt need for methods and materials improving hybridization properties, providing high nuclease resistance and membrane penetration properties. The compounds and compositions of the present invention fulfill this need.

SUMMARY OF THE INVENTION

In one aspect, the present invention relates to a macromolecule, at least a portion of which is of the

structure:



FORMULA I

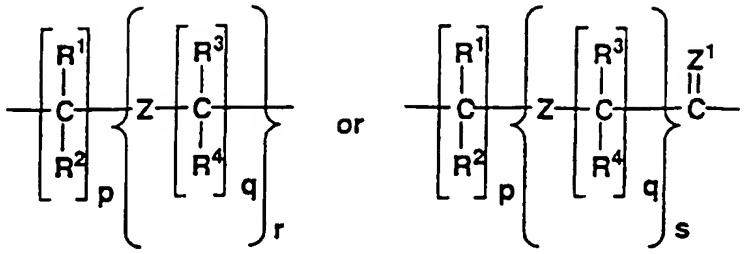
wherein

each B is independently hydrogen, hydroxy, a naturally occurring nucleobase, a non-naturally occurring nucleobase, a DNA intercalator, a covalent or non-covalent DNA-binding group, a heterocyclic moiety, or an aromatic moiety;

each B^1 is independently hydrogen, hydroxy, amino, mercapto, a naturally occurring nucleobase, a non-naturally occurring nucleobase, a DNA intercalator, a covalent or non-covalent DNA-binding group, a heterocyclic moiety, an aromatic moiety, a targeting group, a carrier, a reporter group, or a soluble or non-soluble polymer;

n is an integer from 1 to 50;

each X is independently a single bond, methylene, methylenecarbonyl, C_7-C_{12} aralkylene or substituted aralkylene, C_7-C_{12} aralkylenecarbonyl or substituted aralkylenecarbonyl, or a group of the formula:



wherein

each Z is independently a single bond, O, S, NR^6, $C(=O)NR^6$ or $C(=O)NR^6$;

each Z^1 is independently O, S, NR^5, methylene, or $C(CH_3)_2$;

each of p, q, r, and s is independently an integer from 0 to 20;

each of R^1, R^2, R^3 and R^4 is independently hydrogen; C_1-C_8 alkyl, which may be hydroxy-, or alkoxy-, or alkylthio-substituted; hydroxy; alkoxy; alkylthio; amino or halogen;

each of R^5 and R^6 is independently hydrogen; C_1-C_8 alkyl, which may be hydroxy-, or alkoxy-, or alkylthio-substituted; hydroxy; alkoxy; alkylthio; or amino;

each of Q^1 or Q^2 independently comprises at least three atoms, at least one of which is carbon;

each V is independently oxygen, sulfur, NR^8 or methylene; and

each J is independently hydrogen, azido, halogen, $-OR^7$, $-R^7$ or $-NR^7R^8$, wherein each R^7 is independently $-NR^8R^9$ or R^8, wherein each of R^8 or R^9 is independently hydrogen, C_3-C_{10} branched alkyl or substituted alkyl, C_1-C_{10} unbranched alkyl or substituted alkyl, C_1-C_{10} unbranched oxaalkyl or substituted oxaalkyl, C_6-C_{10} aryl or substituted aryl, C_7-C_{12} aralkyl or substituted aralkyl, C_1-C_{10} unbranched aminoalkyl or substituted unbranched aminoalkyl; C_1-C_{10} unbranched aminooxaalkyl or substituted unbranched aminooxaalkyl, C_3-C_{10} and N_1-N_4 branched (polyamino- or

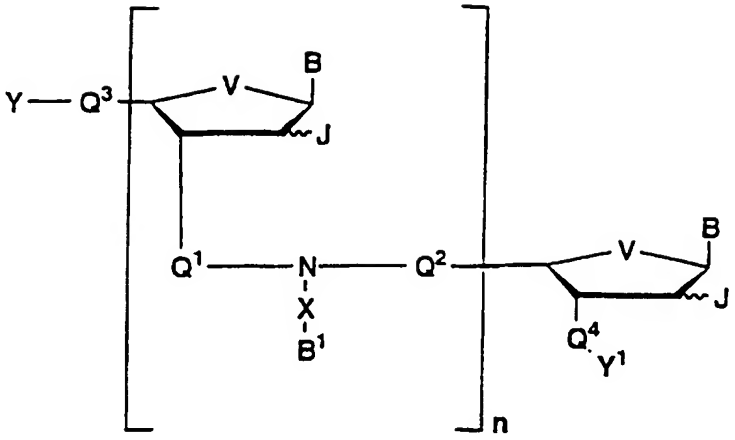
polyaza-)alkyl or substituted (polyamino- or polyaza-) alkyl, C_1-C_{10} and N_1-N_4 unbranched (polyamino- or polyaza-) alkyl or substituted (polyamino- or polyaza-)alkyl, C_1-C_{10} and N_1-N_4 unbranched (polyamino- or polyaza-)oxaalkyl or substituted unbranched (polyamino- or polyaza-)oxaalkyl, a natural or non-natural amino acid side chain radical, or a protecting group.

In another aspect, the present invention relates to pharmaceutical compositions comprising an effective amount of a compound above, and a pharmaceutically suitable carrier.

In another aspect, the present invention relates to methods for the treatment of diseases caused by pathogenic organisms, which comprises administering to a host organism in need of such treatment an effective amount of a compound or pharmaceutical composition described above. The host organism may be any organism in need of such treatment, and includes mammals and humans.

In a further aspect, the present invention relates to methods for the treatment of tumors, which comprises administering to an organism in need of such treatment an effective amount of a compound or pharmaceutical composition described above. The organism may be any organism in need of such treatment, and includes mammals and humans.

In another aspect, the present invention relates to a compound having the formula:



FORMULA II

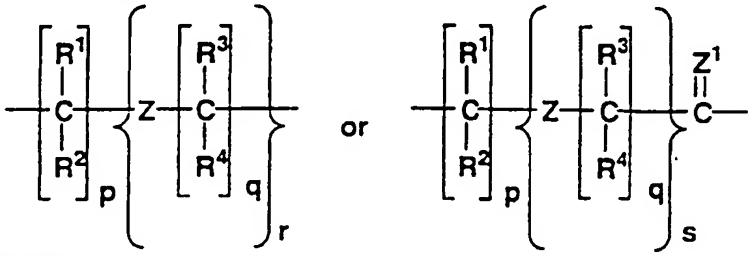
wherein

each B is independently a naturally occurring nucleobase, a non-naturally occurring nucleobase, a heterocyclic moiety, or an aromatic moiety, any of which optionally contains a protecting group;

each B^1 is independently hydrogen, hydroxy, amino, mercapto, a naturally occurring nucleobase, a non-naturally occurring nucleobase, a DNA intercalator, a covalent or non-covalent DNA-binding group, a heterocyclic moiety, or an aromatic moiety, any of which optionally contains a protecting group;

n is an integer from 1 to 50;

each X is independently an optionally protected group selected from a single bond, methylene group, methylenecarbonyl, C_7-C_{12} aralkylene or substituted aralkylene, C_7-C_{12} aralkylenecarbonyl or substituted aralkylenecarbonyl or a group of the formula:



wherein

each Z is independently a single bond, O, S, NR^6, $C(=O)NR^6$, $C(=S)NR^6$, $S(=O)NR^6$, or $S(=O)_2NR^6$;

each Z^1 is independently O, S, Se, NR^5, methylene, or $C(CH_3)_2$;

each of p, q, r, and s is independently an integer from 0 to 20;

each of R^1, R^2, R^3 and R^4 is independently hydrogen; C_1-C_8 alkyl, which may be hydroxy-, or alkoxy-, or alkylthio-substituted; hydroxy; alkoxy; alkylthio; amino or halogen;

each of R^5 and R^6 is independently hydrogen; C_1-C_8 alkyl, which may be hydroxy-, or alkoxy-, or alkylthio-substituted; hydroxy; alkoxy; alkylthio; or amino;

each of Q^1 or Q^2 independently comprises at least three atoms, at least one of which is carbon;

each V is independently oxygen, sulfur, NR^8 or methylene, wherein R^8 is independently hydrogen, C_3-C_{10} branched alkyl or substituted alkyl, C_1-C_{10} unbranched alkyl or substituted alkyl, C_1-C_{10} unbranched oxaalkyl or substituted oxaalkyl, C_6-C_{10} aryl or substituted aryl, C_7-C_{12} aralkyl or substituted aralkyl, C_1-C_{10} unbranched aminoalkyl or substituted unbranched aminoalkyl; C_1-C_{10} unbranched aminooxaalkyl or substituted unbranched aminooxaalkyl, C_3-C_{10} and N_1-N_4 branched (polyamino- or polyaza-)alkyl or substituted (polyamino- or polyaza-)alkyl, C_1-C_{10} and N_1-N_4 unbranched (polyamino- or polyaza-)alkyl or substituted (polyamino- or polyaza-)alkyl, C_1-C_{10} and N_1-N_4 unbranched (polyamino- or polyaza-)oxaalkyl or substituted unbranched (polyamino- or polyaza-)oxaalkyl, a natural or non-natural amino acid side chain radical, or a protecting group;

each J is independently, hydrogen, OR^7, halogen, azide or R^7, any of which is optionally protected, wherein

each R^7 is independently $-NR^8R^9$ or R^8, wherein R^9 is independently hydrogen, C_3-C_{10} branched alkyl or substituted alkyl, C_1-C_{10} unbranched alkyl or substituted alkyl, C_1-C_{10} unbranched oxaalkyl or substituted oxaalkyl, C_6-C_{10} aryl or substituted aryl, C_7-C_{12} aralkyl or substituted aralkyl, C_1-C_{10} unbranched aminoalkyl or substituted unbranched aminoalkyl; C_1-C_{10} unbranched aminooxaalkyl or substituted unbranched aminooxaalkyl, C_3-C_{10} and N_1-N_4 branched (polyamino- or polyaza-)alkyl or substituted (polyamino- or polyaza-) alkyl, C_1-C_{10} and N_1-N_4 unbranched (polyamino- or polyaza-) alkyl or substituted (polyamino- or polyaza-) alkyl, C_1-C_{10} and N_1-N_4 unbranched (polyamino- or polyaza-) oxaalkyl or substituted unbranched (polyamino- or polyaza-) oxaalkyl, a natural or non-natural amino acid side chain radical, or a protecting group;

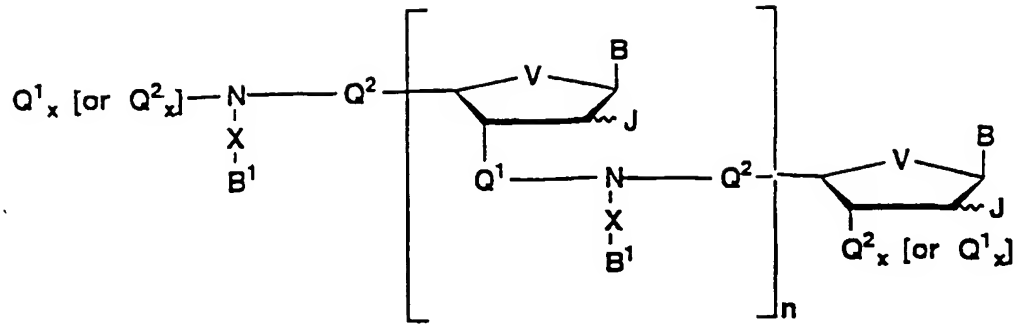
each Q^3 is independently -OX-, -SX- or $-NR^8X-$, any of which is optionally protected;

each Q^4 is independently oxygen, sulfur or NR^8, any of which is optionally protected;

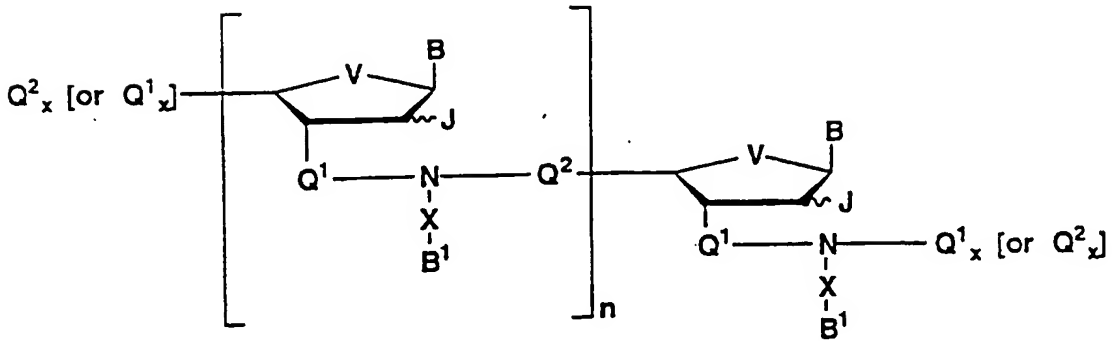
Y is a protecting group;

Y^1 is a spacer group linked to a solid support.

In yet another aspect, the present invention relates to a compound represented by the formula:



or



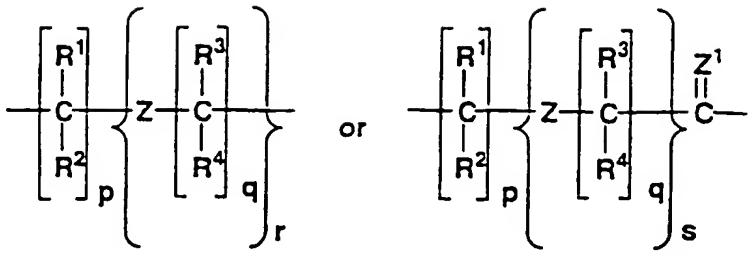
wherein:

each B is independently a naturally occurring nucleobase, a non-naturally occurring nucleobase, a heterocyclic moiety, or an aromatic moiety, any of which optionally contains a protecting group;

each B^1 is independently hydrogen, hydroxy, amino, mercapto, a naturally occurring nucleobase, a non-naturally occurring nucleobase, a DNA intercalator, a covalent or non-covalent DNA-binding group, a heterocyclic moiety, or an aromatic moiety, any of which optionally contains a protecting group;

n is an integer from 1 to 50;

each X is independently one of the following optionally protected groups: a single bond, methylene, methylenecarbonyl, C_7-C_{12} aralkylene or substituted aralkylene, C_7-C_{12} aralkylenecarbonyl or substituted aralkylenecarbonyl or a group of formula:



wherein

each Z is independently a single bond, O, S, NR^6, $C(=O)NR^6$, $C(=S)NR^6$, $S(=O)NR^6$, or $S(=O)_2NR^6$;

each Z^1 is independently O, S, Se, NR^5, methylene, or $C(CH_3)_2$;

each of p, q, r and s is independently an integer from 0 to 20;

each of R^1, R^2, R^3 and R^4 is independently hydrogen; C_1-C_8 alkyl, which may be hydroxy-, or alkoxy-, or alkylthio-substituted; hydroxy; alkoxy; alkylthio; amino or halogen;

each of R^5 and R^6 is independently hydrogen; C_1-C_8 alkyl, which may be hydroxy-, or alkoxy-, or alkylthio-substituted; hydroxy; alkoxy; alkylthio; or amino;

each of Q^1 or Q^2 comprises at least three atoms, at least one of which is carbon;

each V is independently oxygen, sulfur, NR^8 or methylene;

each J is independently one of the following optionally protected groups: hydrogen, OR^7, halogen, azide or R^7, wherein each R^7 is independently $-NR^8R^9$ or R^8, wherein each of R^8 or R^9 is independently hydrogen, C_3-C_{10} branched alkyl or substituted alkyl, C_1-C_{10} unbranched alkyl or substituted alkyl, C_1-C_{10} unbranched oxaalkyl or substituted oxaalkyl, C_6-C_{10} aryl or substituted aryl, C_7-C_{12} aralkyl or substituted aralkyl, C_1-C_{10} unbranched aminoalkyl or substituted unbranched aminoalkyl; C_1-C_{10} unbranched

aminooxaalkyl or substituted unbranched aminooxaalkyl, C_3-C_{10} and N_1-N_4 branched (polyamino- or polyaza-)alkyl or substituted (polyamino- or polyaza-)alkyl, C_1-C_{10} and N_1-N_4 unbranched (polyamino- or polyaza-)alkyl or substituted (polyamino- or polyaza-)alkyl, C_1-C_{10} and N_1-N_4 unbranched (polyamino- or polyaza-)oxaalkyl or substituted unbranched (polyamino- or polyaza-)oxaalkyl, a natural or non-natural amino acid side chain radical, or a protecting group;

each of Q^1_x and Q^2_x, comprising at least one atom, is independently selected from optionally protected or activated fragments of Q^1 or Q^2.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1-8 each depicts comparisons of prior art compounds (labeled (A) in each Figure) with various compounds of the present invention, labeled (B-1) through (B-24) and (C-1) through (C-6).

Figures 9-11 each depicts compounds of the present invention.

Figures 12-14 each depicts comparisons of prior art compounds (labeled (A) in each Figure) with various compounds of the present invention, labeled (E-1) through (E-13).

Figure 15 depicts compounds of the present invention.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is directed to macromolecules that are able to function like oligonucleotides and which also possess other useful properties. As is illustrated in the Examples and Schemes of this specification, the macromolecules are constructed from basic nucleoside units and specific linker units bearing nucleobase or other nucleic acid-binding elements. The nucleoside units, joined by a nucleobase bearing linker of the present invention (*i.e.*, the Q^1-N-Q^2 segment of Formula I), forms trimeric units. The trimeric units can be further extended

to pentameric, heptameric and other, higher order macromolecules by addition of further nucleosides. The trimeric units (and/or the higher order units) can be connected via linkages other than those of the invention, as for example, via a normal phosphodiester linkage, a phosphothioate linkage, a phosphodithioate linkage, a phosphoroamidate linkage, a phosphotriester linkage, a methyl or other alkylphosphonate linkage or other linkage. The linkage of the present invention contains two parts (or sublinkages, *i.e.*, Q^1 and Q^2 in Formula I) separated by an aza-nitrogen atom. The aza-nitrogen atom is an achiral site of attachment of a nucleobase or any other nucleic acid binding moiety. In certain embodiments a single type of sublinkage is used to join nucleosides and aza-nitrogen atom(s). In other embodiments, two different sublinkages are used to form trimeric units, or two or more different sublinkages are used to form the higher order units. Within the same macromolecule of the invention, sublinkages of different units (trimeric or higher order units) may be the same or of different types as described in this specification. Some trimeric units (and/or the higher order units) carry naturally occurring or non-naturally occurring nucleic bases attached to an achiral aza-nitrogen atom within an internucleosidic linkage, other units carry a nucleobase-binding ligand other than a nucleobase, described below more fully.

The naturally occurring nucleobases which are referred to herein include the four main naturally occurring nucleobases, *i.e.* thymine, cytosine, adenine or guanine, or other naturally occurring nucleobases, *e.g.* hypoxanthine, uracil, thiouracil, 5-methylcytosine, *etc.*

The non-naturally occurring nucleobases which are referred to herein include, for example, fluorouracil, bromovinyluracil, triazolcarboxamide, benzimidazole, *etc.*

The heterocyclic moieties which are referred to herein include any heterocycle containing at least one heteroatom fused or non-fused ring systems, for example, nitroindole

derivatives, nitroimidazole derivatives, nitrotriazole derivatives, *etc.*

The DNA-binding groups which are referred to herein include: 1) covalent DNA-binding groups, which interact with DNA by means of chemical modification (for example, mustard gas derivatives, psoralen and its derivatives, mitomycin C, *etc.*); 2) non-covalent DNA-binding groups, which interact with DNA by means of hydrogen bond formation, intercalation, electrostatic forces, *etc.* Hydrogen bond formation between functional groups of DNA (or RNA) and functional groups of nucleic acid binding ligands plays an important role in specific recognition of nucleic acids (especially double stranded nucleic acids) by antibiotics (*e.g.*, distamycin, netropsin, echinomycin, *etc.*), or proteins (*e.g.*, repressors, restrictases, *etc.*), or oligonucleotides (*e.g.*, DNA double strand formation, or DNA triple strand formation). Intercalation is a special kind of stacking observed in nucleic acids which is associated with separation of two adjacent base pairs to allow insertion of a planar aromatic (hetero)-cyclic group, known as the intercalator. The intercalators capable of binding to double stranded nucleic acids which are referred to herein include, for example, acridine or its derivatives, phenanthridine or its derivatives, *etc.* The intercalators capable of binding to preferably triple stranded clusters of nucleic acids (compare to duplexes) which are referred to herein include, for example, coralyne (a member of the protoberberine family of alkaloids), propidium bromide, *etc.*

The carriers which are referred to herein include, for example, a polyamine group (*e.g.*, polyethyleneimine, spermine, spermidine, poly-L-lysine, starburst dendrimers, *etc.*), or lipophilic groups (*e.g.*, cholesterol, alkyl chain, *etc.*), or a soluble polymer (*e.g.*, polyethylene glycols, polysaccharides, proteins, *etc.*), or by a non-soluble polymer (*e.g.*, dextranes, polyacrylamide derivatives, polyvinyl derivatives, *etc.*), or a targeting

ligand (*e.g.*, sugar or sugar phosphate residues which act as binding sites to receptors on the surface of target cell, antibodies, immunoglobulins, *etc.*).

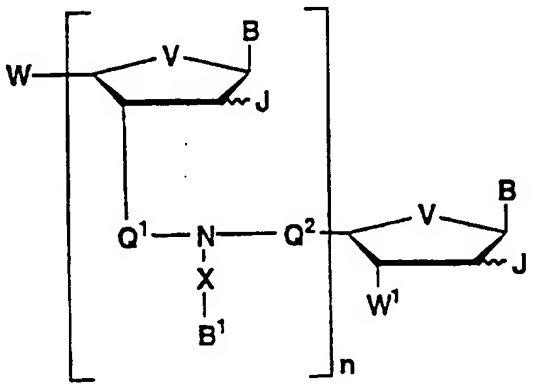
As used herein, "nucleoside" refers to a unit composed of a heterocyclic base and a sugar, and includes the natural occurring nucleosides, including 2'-deoxy and 2'-hydroxyl forms, *e.g.* as described in Kornberg and Baker, DNA Replication, 2nd Ed. (Freeman, San Francisco, 1992). In naturally occurring nucleosides, the heterocyclic base typically is adenine, cytosine, guanine, thymine or uracil; the sugar is normally deoxyribose, *i.e.*, erythro-pentofuranosyl, or ribose, *i.e.*, ribo-pentofuranosyl. "Analogues" in reference to nucleosides includes synthetic nucleosides having modified base moieties (for example, 5(6)-nitroindole, 4-nitrotriazole, 3(4)-nitrobenzimidazole, 2-aminopurine, benzimidazole, 5-fluorouracil, and the like) and/or modified sugar moieties (for example, arabino, xylo or lyxo pentafuranosyl sugars; or substituted arabino, erythro, ribo, xylo or lyxo pentafuranosyl sugars; or acyclic moieties mimicking sugar; or hexose sugars; *etc.*) *e.g.* described by Scheit, Nucleotide Analogues (John Wiley, New York, 1980). Such analogues include the natural and synthetic nucleosides with or without an appropriate protecting group for synthesis.

As used herein, "nucleotide" refers to a nucleoside having a phosphate group esterified to at least one of the sugar hydroxyl groups.

The term "oligonucleotide" as used herein includes linear oligomers of natural or modified nucleosides, including deoxyribonucleosides, ribonucleosides, alpha-anomeric forms thereof, and the like, usually linked by phosphodiester bonds or analogues thereof ranging in size from a few monomeric units, *e.g.* 2-3, to several hundreds of monomeric units. "Analogues" in reference to oligonucleotide refers to structures including modified portions such as modified sugar moieties, modified base moieties or modified sugar linking moieties. Exemplary

among these are phosphorothioate, phosphorodithioate, phosphoroamidate, phosphoroanilidate, phosphodiester internucleoside linkages used in place of phosphodiester internucleoside linkages; deaza or aza purines and pyrimidines may be used in place of natural purines or pyrimidines; pyrimidine bases having a substituent group at, for example, the 4-, 5- or 6-positions; purine bases having altered or replacement substituent groups at, for example, the 2-, 6- or 8-positions; or sugars having substituent groups at their 2'-position, substitutions for one or more of the hydrogen atoms of the sugar, or carbocyclic sugar. Preferably, oligonucleotides of the present invention are oligomers of the natural nucleosides having a length in the range of 2 to 50, and more preferably, having a length in the range of 2 to 20 monomeric units.

As mentioned above, the compounds of the present invention are represented by an oligonucleotide, or modified oligonucleotide, or so-called "oligonucleoside" (*i.e.* phosphate-free oligonucleotide) optionally modified at the ends, at least a portion of which has the structure of formula **Ia**:



Formula **Ia**

In Formula Ia, substituents W and W^1 represent the remainder of the macromolecule. W and W^1 may be any substituents which do not detract from the utility of the

present compounds. Preferably, each of W and W^1 are independently -H; -OH; optionally modified phosphate or phosphate analogs; nucleosides or analogs thereof; nucleotides or analogs thereof; oligonucleotides or analogs thereof; amino; mercapto; a DNA intercalator; a covalent or non-covalent DNA-binding group; a heterocyclic moiety; or an aromatic moiety, any of which optionally contains a protecting group; peptides or analogs thereof; chelating groups (*e.g.*, EDTA); polymers (*e.g.* polyamides, polycations, polyanions, *etc.*) sugars; saccharides; polysaccharides; or lipophilic groups.

Preferably, each B independently comprises naturally occurring nucleobases, non-naturally occurring nucleobases, heterocyclic moieties, aromatic moieties, DNA intercalators, covalent or non-covalent DNA-binding groups; more preferably each B is a naturally occurring nucleobase or non-naturally occurring nucleobase. The most preferred choice for each B is a naturally occurring nucleobase.

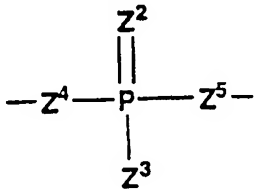
In certain preferred embodiments, at least one of B^1 is a naturally occurring nucleobase; in other preferred embodiments at least one of B^1 is a non-naturally occurring nucleobase; in other preferred embodiments at least one of B^1 is a DNA intercalator (such as acridine derivatives, phenazine derivatives, *etc.*); in other preferred embodiments at least one of B^1 is a covalent DNA-binding group (such as mustard gas derivatives, psoralen derivatives, *etc.*); in other preferred embodiments at least one of B^1 is a DNA-binding antibiotic (such as daunomycin, actinomycin D or another representative of the actinomycin family, netropsin and its derivatives, distamycin and its derivatives, *etc.*); in other preferred embodiments at least one of B^1 is a reporter group (such as a fluorescent or chemiluminescent label, biotin, *etc.*); in other preferred embodiments at least one of B^1 is a targeting group for recognition of definite cells (such as an antibody or saccharide); in other preferred embodiments at least one of

B^1 is a soluble or non-soluble polymer; in other preferred embodiments at least one of B^1 is a reactive functional group suitable for postsynthetic modification of an oligonucleotide (such as amino, mercapto, aldehyde, carboxyl, *etc.*).

In a preferred embodiment, n is from 1 to 20; most preferably n=1.

In certain preferred embodiments, when B^1 is a naturally occurring nucleobase or a non-naturally occurring nucleobase, X comprises from 1 to 4 atoms; in other preferred embodiments, when B^1 is selected from DNA intercalators, covalent or non-covalent DNA-binding groups, or DNA-binding antibiotics, X^1 comprises from 1 to 12 atoms; in a most preferred embodiment, when B^1 is a nucleobase, X^1 is methylenecarbonyl.

In preferred embodiments, each of Q^1 and Q^2 independently contains at least one of the following groups: oxygen; sulfur; substituted carbon; carbonyl; thiocarbonyl; sulfone; sulfoxide; C_1-C_8 alkylene; C_2-C_8 alkenylene; C_2-C_8 alkynylene; C_1-C_8 oxaalkylene or thiaalkylene or azaalkylene each containing one or two different heteroatoms or heteroatoms of the same type; or NR^7, or $^+NR^8R^9$, or $NR^7C(=O)-$, or $-NR^7C(=S)-$, or $-NR^7S(=O)-$, or $-NR^7S(=O)_2-$ in either orientation, wherein R^7, R^8 and R^9 have been specified above; or X, wherein X has been specified above; or a group of:



wherein

each of Z^4 or Z^5 is independently selected from the group consisting of a single bond, O, S, and NR^7, wherein R^7 has been specified above;

each Z^3 is independently selected from the group

consisting of hydrogen, R^8, OR^7, SR^7, and NR^7R^8, wherein R^7 and R^8 have been specified above; and

each Z^2 is independently selected from the group consisting of O, S, and NR^7, wherein R^7 has been specified above.

In certain preferred embodiments each of Q^1 and Q^2 independently contains from 2 to 8 atoms; in more preferred embodiments Q^1 and Q^2 each independently contains from 3 to 6 atoms; most preferably 4 or 5 atoms. Most preferably, each Q^1 is independently selected from the following groups:

$-O-CH_2-CH_2-CH_2-$, $-O-CH_2-C(=O)-NH-$, $-NH-NH-C(=O)-CH_2-$, $-NH-N=CH-CH_2-$, $-NH-NH-CH_2-CH_2-$, $-O-NH-C(=O)-CH_2-$, $-O-N=CH-CH_2-$, $-O-NH-CH_2-CH_2-$, $-CH_2-NH-C(=O)-CH_2-$, $-NH-NH-C(=O)-NH-$, $-O-C(=O)-NH-CH_2-CH_2-$, $-O-P(=Z^2)Z^3-NH-CH_2-CH_2-$, or $-CH_2-NH-CH_2-CH_2-$, in either orientation; and

each Q^2 is independently selected from the following groups: Q^1, $-CH_2-O-CH_2-CH_2-CH_2-$, $-CH_2-O-CH_2-C(=O)-NH-$, $-CH_2-NH-NH-C(=O)-CH_2-$, $-CH_2-NH-N=CH-CH_2-$, $-CH_2-NH-NH-CH_2-CH_2-$, $-CH_2-O-NH-C(=O)-CH_2-$, $-CH_2-O-N=CH-CH_2-$, $-CH_2-O-NH-CH_2-CH_2-$, $-CH_2-NH-C(=O)-CH_2-$, $-CH_2-NH-NH-C(=O)-NH-$, $-O-C(=O)-NH-CH_2-CH_2-$, $-O-P(=Z^2)Z^3-NH-CH_2-CH_2-$, or $-CH_2-CH_2-NH-CH_2-CH_2-$, in either orientation.

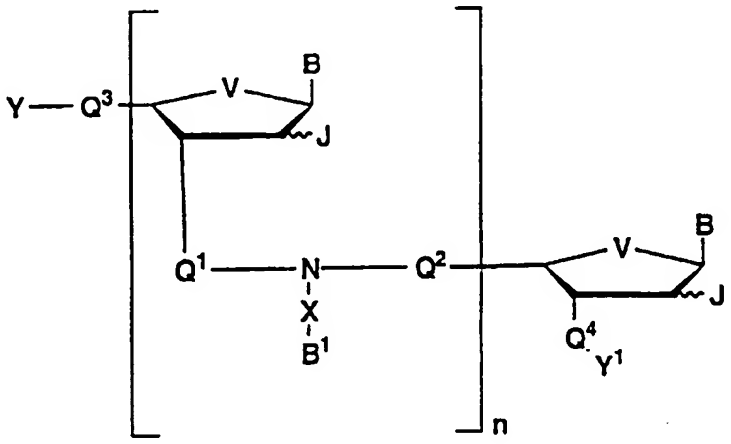
Preferably, at least one of V is oxygen. The most preferred choice for each V is oxygen.

In more preferred embodoments, at least one of J is independently selected from the group of hydrogen, fluorine or OCH_3; the most preferred choice for J is hydrogen.

The compounds of the invention are synthesized by adaptation of standard oligonucleotide synthesis procedures, or by adaptation of standard peptide synthesis procedures, or by a combination of both mentioned

procedures in solution or on solid phase.

Using standard oligonucleotide synthesis procedures, the compounds of the present invention may be prepared by incorporation of fragments of formula I onto the 5'-end of a growing oligonucleotide or modified oligonucleotide chain, represented by the formula II:



Formula II

wherein each of B, B^1, Q^1, Q^2, V and J are as described above, any of which is optionally blocked with a protecting group if appropriate (*e.g.*, acetyl, isobutyryl, phenoxyacetyl, benzoyl, cyanoethyl, 4-nitrophenylethyloxycarbonyl, 4-nitrophenylethyl, benzyloxycarbonyl, p-anisyldiphenylmethyl, di-p-anisylphenylmethyl, pixyl; tert-butyloxycarbonyl, diphenylcarbamoyl, formamidino, acetamidino, trialkylsilyl having from 3 to 14 carbon atoms, 9-fluorenylmethyl carbamate, or the like; see Greene and Wuts, Protective Groups in Organic Synthesis, 2nd Edition (John Wiley, New York, 1991));

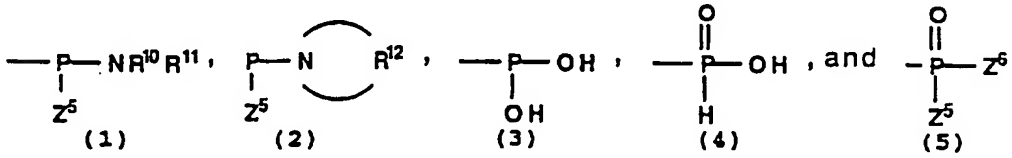
each Q^3 independently comprises a single bond, oxygen, sulfur, $-NR^8-$, $-OX-$, $-SX-$, $-X-$, or $-NR^8X-$, wherein X and R^8 have the meanings specified above in connection with formula I; more preferably each Q^3 independently comprises a single bond, $-OCH_2-$, $-SCH_2-$, $-CH_2-$, $-NR^8CH_2-$, $-CH_2CH_2-$, and

$-OCH_2CH_2-$; the most preferred choice for Q^3 is $-OCH_2-$;

each Q^4 independently comprises a single bond, oxygen, sulfur and NR^8, wherein R^8 has the meanings specified above for formula I;

Y is a protecting group such as triphenylmethyl, p-anisyldiphenylmethyl, di-p-anisylphenylmethyl, pixyl, trialkylsilyl having from 3 to 14 carbon atoms, 9-fluorenylmethyl carbamate, trifluoroacetyl, or the like; more preferably Y is p-anisyldiphenylmethyl, di-p-anisylphenylmethyl, or pixyl;

Y^1 is a spacer group linked to a solid support, which spacer comprises carbonyl, ester, carbamate, urethane, hydrazide, C_1-C_{14} alkylene or modified alkylene, C_6-C_{14} aralkylene or modified aralkylene, C_6-C_{14} alkylarene or modified alkylarene, C_1-C_{100} oxaalkylene or thiaalkylene or azaalkylene each containing from one to fifty different heteroatoms or hetroatoms of the same type, where aza groups are, optionally, protected by amino protecting groups, C_1-C_{14} alkylenecarbonyl or alkylenethiocarbonyl or alkylenesulfone or alkylenesulfoxide, C_1-C_{100} oxaalkylenecarbonyl or thiaalkylenecarbonyl or azaalkylenecarbonyl (or their thiocarbonyl or sulfone or sulfoxide analogues) each containing from one to fifty different heteroatoms or hetroatoms of the same type, where aza groups are, optionally, protected by amino protecting groups; or a group of the formula (III):



Formula (III)

or salts thereof, wherein

each of R^{10} or R^{11} independently comprises C_3-C_{10} branched alkyl, C_1-C_{10} unbranched alkyl or oxaalkyl, C_6-C_{10}

aryl, C_7-C_{12} aralkyl; a more preferred choice for each of R^{10} or R^{11} is C_3-C_5 branched alkyl or C_1-C_4 unbranched alkyl; the most preferred choice for each of R^{10} or R^{11} is isopropyl;

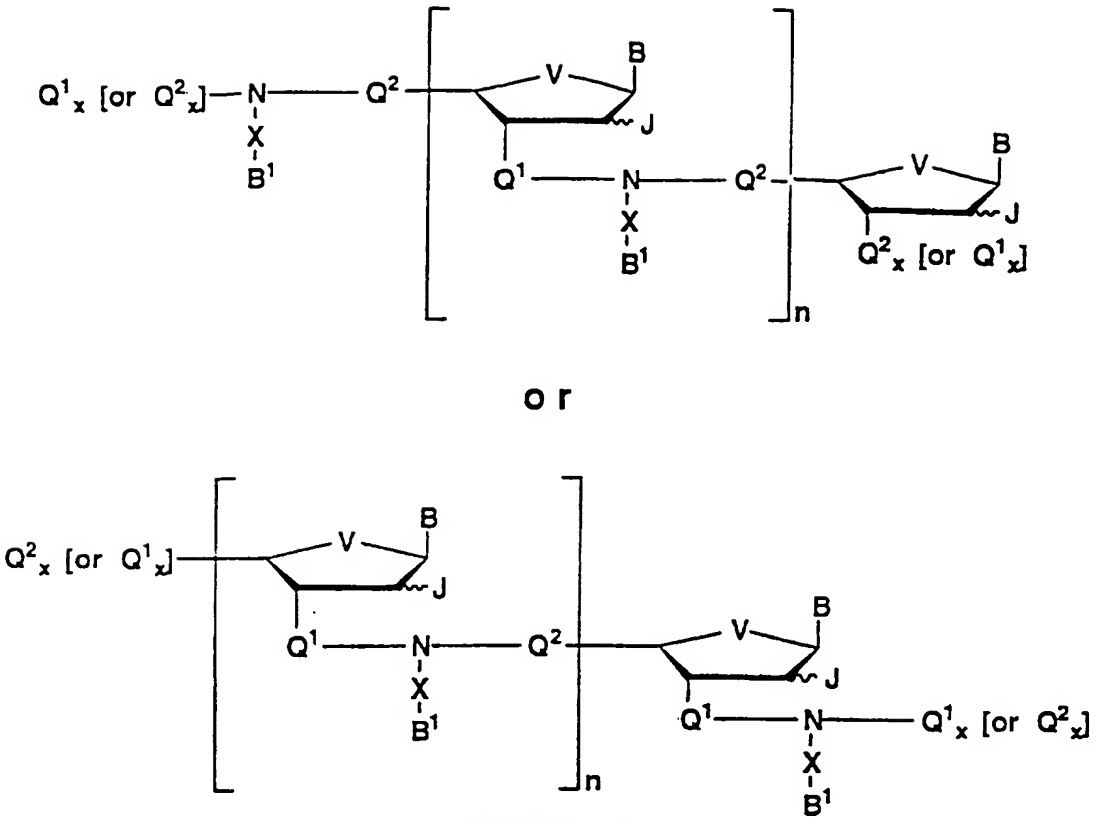
R^{12} is C_2-C_8 alkylene, C_2-C_8 alkenylene or -C_2-C_8 oxaalkylene, comprising one or two heteroatoms; most preferably R^{12} is a morpholino group;

Z^5 is any phosphate protecting group; preferably, 4-Cl-C_6H_4-O-, 2-Cl-C_6H_4-O-, 4-NO_2-$C_6H_4CH_2CH_2$-O-, 2,4-NO_2-$C_6H_3CH_2CH_2$-O-, 2,4-Cl-C_6H_3-O-, 2,3-Cl-C_6H_3-O-, $NCCH_2CH_2$-O-, $NCCH_2C(CH_3)_2$-O-, CH_3O-, $(Z)_3CCH_2$-O-, $R^{10}S$-, $R^{10}SCH_2CH_2$-O-, or $R^{10}SO_2CH_2$-O-, wherein Z is halogen and R^{10} is independently selected from the meanings described above;

Z^6 is -F, -Cl, -Br, -I, imidazol-1-yl, tetrazol-1-yl, 1,2,4-triazol-1-yl and 1-hydroxy-benzotriazol-O-yl; in certain preferred embodiments Y^1 is a functionalized solid support of CPG or polystyrene nature; in other preferred embodiments Y^1 is -HP(=O)OH and salts thereof; in other preferred embodiments Y^1 is -$P(OCH_2CH_2CN)(N(i\text{-}Pr)_2)$ or -$P(OCH_3)(N(i\text{-}Pr)_2)$.

Compounds of Formula (III) are commonly known (with reference to the activable phosphorus group, Y^1) as phosphoroamidites, (III-1 and -2), phosphorous acids (III-3), H-phosphonates (III-4) and activated phosphodiesters (III-5). Structures (III-3) and (III-4) represent moderately strong acids, and the reagents represented by these structures are generally isolated and used as their organically soluble salts.

The novel compounds for incorporation of fragment of formula I onto the 5'-end of a growing modified oligonucleotide chain, represented by the group of formula IV:



Formula IV

wherein each of B, B1, Q^1, Q^2, V, X and J are as described above, any of which is optionally blocked with a protecting group;

each of Q^1_x and Q^2_x comprises optionally protected or activated fragments of Q^1 or Q^2; more preferably each of Q^1_x and Q^2_x contains from one to three atoms, any of which optionally contains a protecting group; in most preffered embodiments each Q^1_x independently comprises Y^2-NH-CH_2-, Y^2-NH-O-, Y^2-NH-NH-, Y^2-NH-, Y^2-NH-CH_2-CH_2-, Y^2-NH-NH-CH_2-, Y^2-NH-C(=O)-CH_2-, Y^2-NH-C(=O)-NH-, Y^2-NH-NH-C(=O)-, wherein Y^2 is a protecting group or hydrogen; more preferably Y^2 comprises p-anisyldiphenylmethyl, di-p-anisylphenylmethyl, pixyl, 9-fluorenylmethyl carbamate, or trifluoroacetyl; and each Q^2_x independently comprises E-CH_2-CH_2-, E-CH_2-, E-NH-,

E-O-, wherein E is independently halogen, aldehyde, acetal, $-S(=O)_2R^{13}$, or $-COR^{13}$, wherein R^{13} is a hydroxyl or activated group; more preferably R^{13} is selected from halogen, hydroxyl, pentafluorophenoxy, tetrafluorophenoxy, p-nitrophenoxy, or N-succinimidoxy.

The compounds of the present invention may be divided into five groups:

(1) oligonucleotide analogues, wherein the non-nucleosidic linkage bears a nucleic base;

(2) oligonucleotide analogues, wherein the non-nucleosidic linkage bears a covalent DNA-binding group, or a non-covalent DNA-binding group, or a DNA-intercalator, or a DNA-binding antibiotic;

(3) oligonucleotide analogues, wherein the non-nucleosidic, preferrably non-phosphate containing linkage, is used as a new type of linker connecting clusters of oligonucleotides or modified oligonucleotides;

(4) oligonucleotide analogues, wherein the non-nucleosidic linkage bears a carrier, or polymer, or targeting ligand, or lipophilic group; and

(5) oligonucleotide analogues, wherein the non-nucleosidic linkage bears a reporter group, such as biotin.

The oligonucleotide analogues (1)-(5) are able to recognize both single stranded and double stranded nucleic acids. The oligonucleotide analogues (1) with fragments of formula I placed on the 3'- and/or 5'-end or distributed along the oligonucleotide chain demonstrate the ability to bind DNA fragments and at the same time possess increased enzymatic stability. The oligonucleotide analogues (2) with fragments of formula I placed in definite positions along the oligonucleotide chain bind efficiently to double stranded DNA or RNA, and attachment of a covalent DNA-binding group, or a non-covalent DNA-binding group, or a DNA-intercalator, or a DNA-binding antibiotic, provides another opportunity to overcome the problem of recognition of polypyrimidine tracts by triplex forming

oligonucleotides (TFO). The oligonucleotide analogues (3) can be used as a new type of TFO. The oligonucleotide analogues (4) and (5) are useful in the synthesis of oligonucleotide conjugates.

The improved enzymatic stability and binding ability and cell membrane penetration of the compounds of the invention render them efficient as antisense (binding to RNA) or antigene (binding to DNA) agents.

Also, the invention provides reagents and methods for inhibiting transcription and/or replication of particular genes or for degradation of particular regions of double stranded DNA in cells of an organism by administering to said organism a compound of invention as defined above.

Further, the invention provides reagents and methods for killing or mutating cells (such as tumor cells) or pathogenic organisms (such as viruses, bacteria, fungi, *etc.*) by contacting said cells or organisms with compounds or compositions of the present invention which have specificity for such cells or organisms.

Viruses susceptable to treatment according to the present invention would be readily determined by one of ordinary skill, and could include herpes simplex virus (HSV), human papillomavirus (HPV), human immunodeficiency virus (HIV), *etc.*

For therapeutic or prophylactic treatment, the compounds of the present invention may be formulated in a pharmaceutical composition, which may include, in addition to an effective amount of active ingredient, pharmaceutically acceptable carriers, thickeners, diluents, buffers, preservatives, surface active agents and the like. Pharmaceutical compositions may also include one or more other active ingredients such as antimicrobial agents, antiinflammatory agents, and the like.

The pharmaceutical compositions of the present invention may be administered in a number of ways as will be apparent to one of ordinary skill. Administration may be done topically, orally, by inhalation, or parenterally,

for example.

Topical formulations may include ointments, lotions, creams, gels, drops, suppositories, sprays, liquids and powders. Oral formulations include powders, granules, suspensions or solutions in water or non-aqueous media, capsules or tablets, for example. Thickeners, flavorings, diluents, emulsifiers, dispersing aids or binders may be used as needed.

Parenteral formulations may include sterile aqueous solutions which may also contain buffers, diluents and other suitable additives.

The dose regimen will depend on a number of factors which may readily be determined, such as severity and responsiveness of the condition to be treated, but will normally be one or more doses per day, with a course of treatment lasting from several days to several months, or until a cure is effected or a diminution of disease state is achieved. One of ordinary skill may readily determine optimum dosages, dosing methodologies and repetition rates. In general, it is contemplated that unit dosage form compositions according to the present invention will contain from about 0.01 mg to about 100 mg of active ingredient, preferably about 0.1 mg to about 10 mg of active ingredient. Topical formulations (such as creams, lotions, solutions, *etc.*) may have a concentration of active ingredient of from about 0.01% to about 50%, preferably from about 0.1% to about 10%.

The following Figures and Schemes illustrate, but not limit, the compounds and utilities of the present invention. Figures I-VI represent some trimeric fragments of the present invention with the variations in structures of linking moieties Q_1 and Q_2 (see Formula I of present Description). All depicted structures from B-1 (Fig. 1) through B-24 (Fig. 6) represent some oligonucleoside trimeric fragments of the invention with variations in the linking moieties, wherein B^n is preferably a nucleic base.

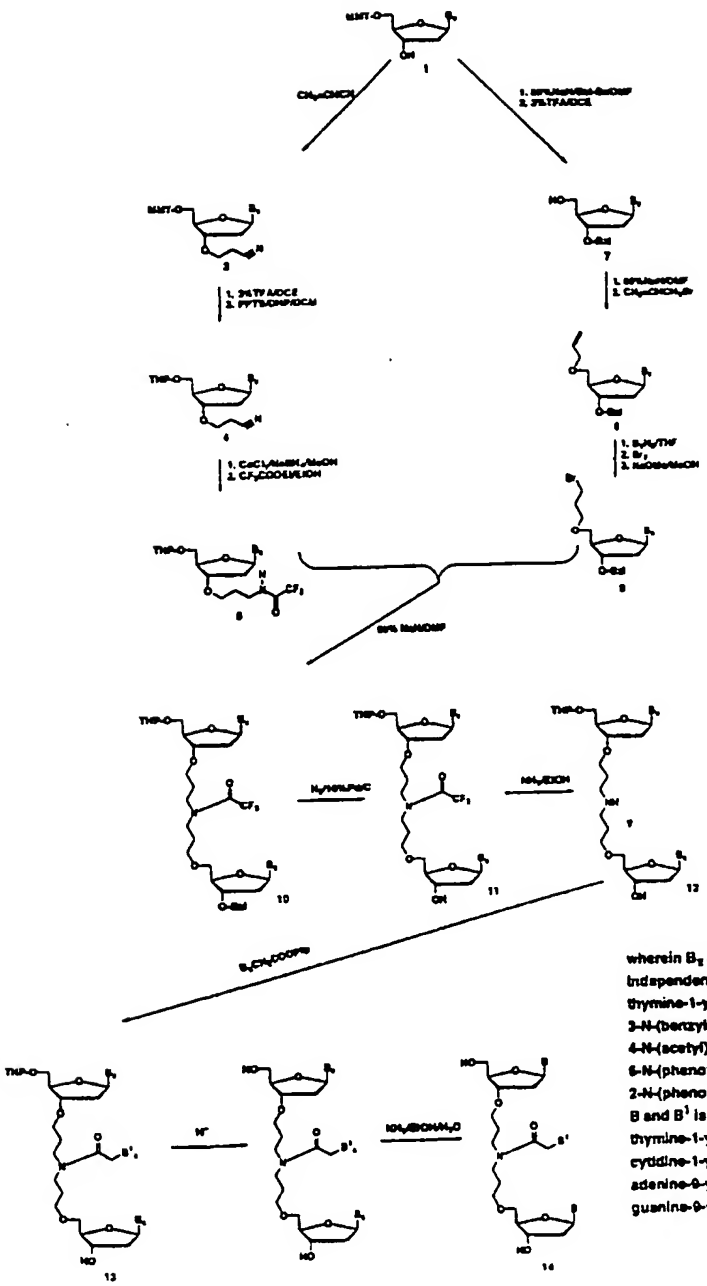
The structures C-1 (Fig. 7) through C-9 (Fig. 9) represent examples wherein the aza nitrogen atom in Q_1-N-Q_2 serves as a site of attachment for an intercalating moiety. Formula C-1 (Fig. 7) illustrates the case where an acridine residue is attached through an acyl-type spacer; in formula C-2 the acridine residue is attached through an alkyl spacer. In both structures the intercalator is bound to the N-atom in propyliminopropyl internucleoside linking moiety. The structures from C-3 through C-6 (Fig. 8) demonstrate examples of attachment of an intercalator to the aza-atom in a relatively long (20-30 atoms) phosphate-free internucleoside linking moiety. Figure 9 shows the structures of the present invention with an attached coralyne residue. As was shown (J.S. Lee, *Biochemistry*, **1993**, v.32, pp. 5591-5597), coralyne demonstrates considerable preference for triplex binding (binding constants are about 10^6 M^{-1}) compared to duplex binding (binding constants are about 10^3-10^4 M^{-1}). Coralyne conjugated to a triple helix forming oligonucleotide can be favorable for self-stabilization of the formed triplex.

Figures 10 and 11 represent situations where a DNA covalent binding moiety (*i.e.*, psoralen) is conjugated to a internucleoside linker of the present invention. Psoralen binds preferentially to double-stranded DNA molecules and attachment of psoralen to an abasic site within the triplex forming oligonucleotide can considerably increase accuracy of site modification of the DNA duplex.

Figures 12-14 demonstrate examples of trimeric units of the present invention bearing a functional group attached to the aza-nitrogen. These functional groups are used as a site for postsynthetic modification of oligonucleotides with DNA-active substances (such as intercalators, alkylators, DNA-binding antibiotics, or any other nucleic acid binding group as described above).

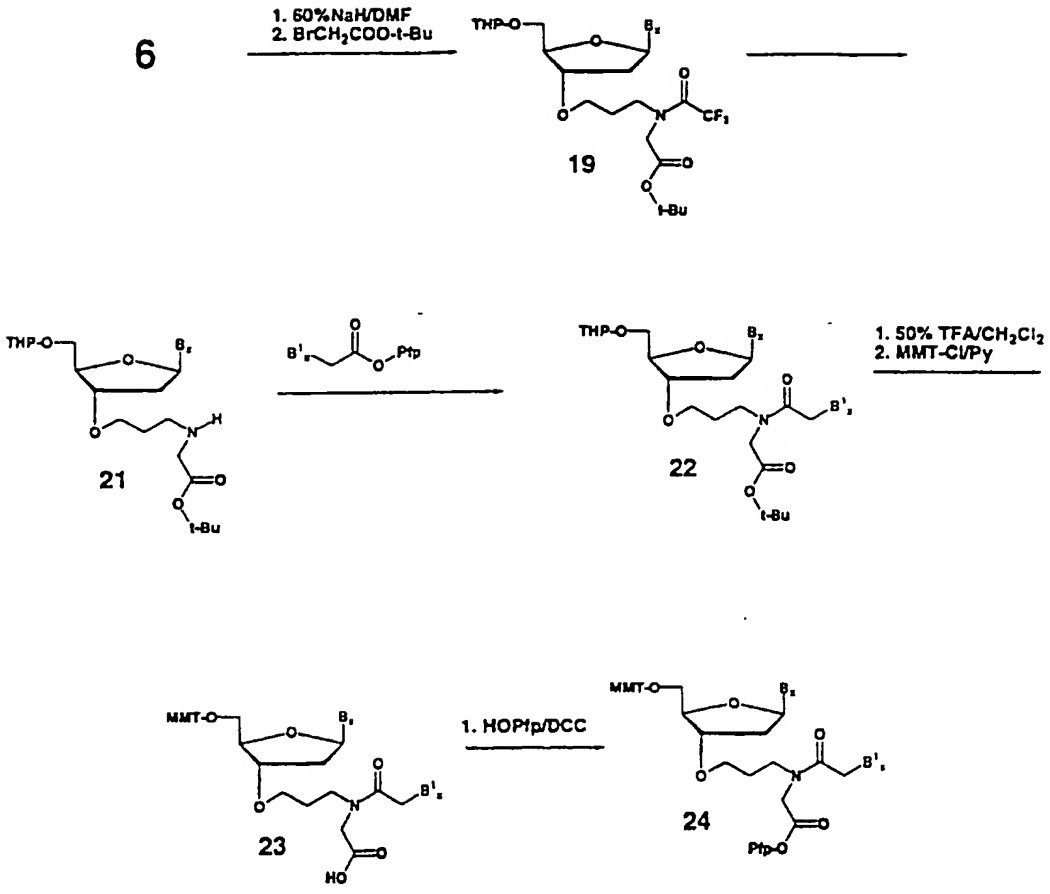
Various methods for the synthesis of compounds according to the present invention are summarized in the following schemes, which are described in detail below.

Scheme I



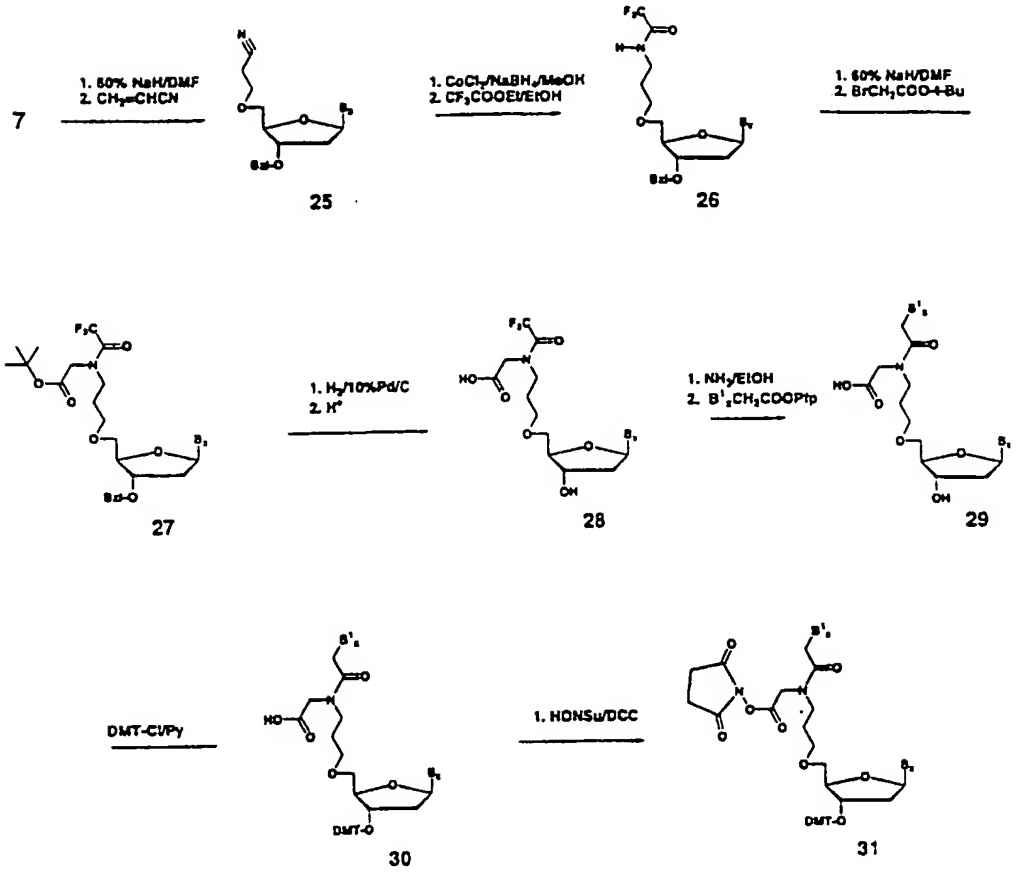
wherein B_x and B^1_x is selected independently from:
thymine-1-yl;
3-N-(benzyloxymethyl)thymine-1-yl;
4-N-(acetyl)cytidine-1-yl;
6-N-(phenoxyacetyl)adenine-9-yl;
2-N-(phenoxyacetyl)guanine-9-yl;
B and B^1 is selected independently from:
thymine-1-yl;
cytidine-1-yl;
adenine-9-yl;
guanine-9-yl;

Scheme II

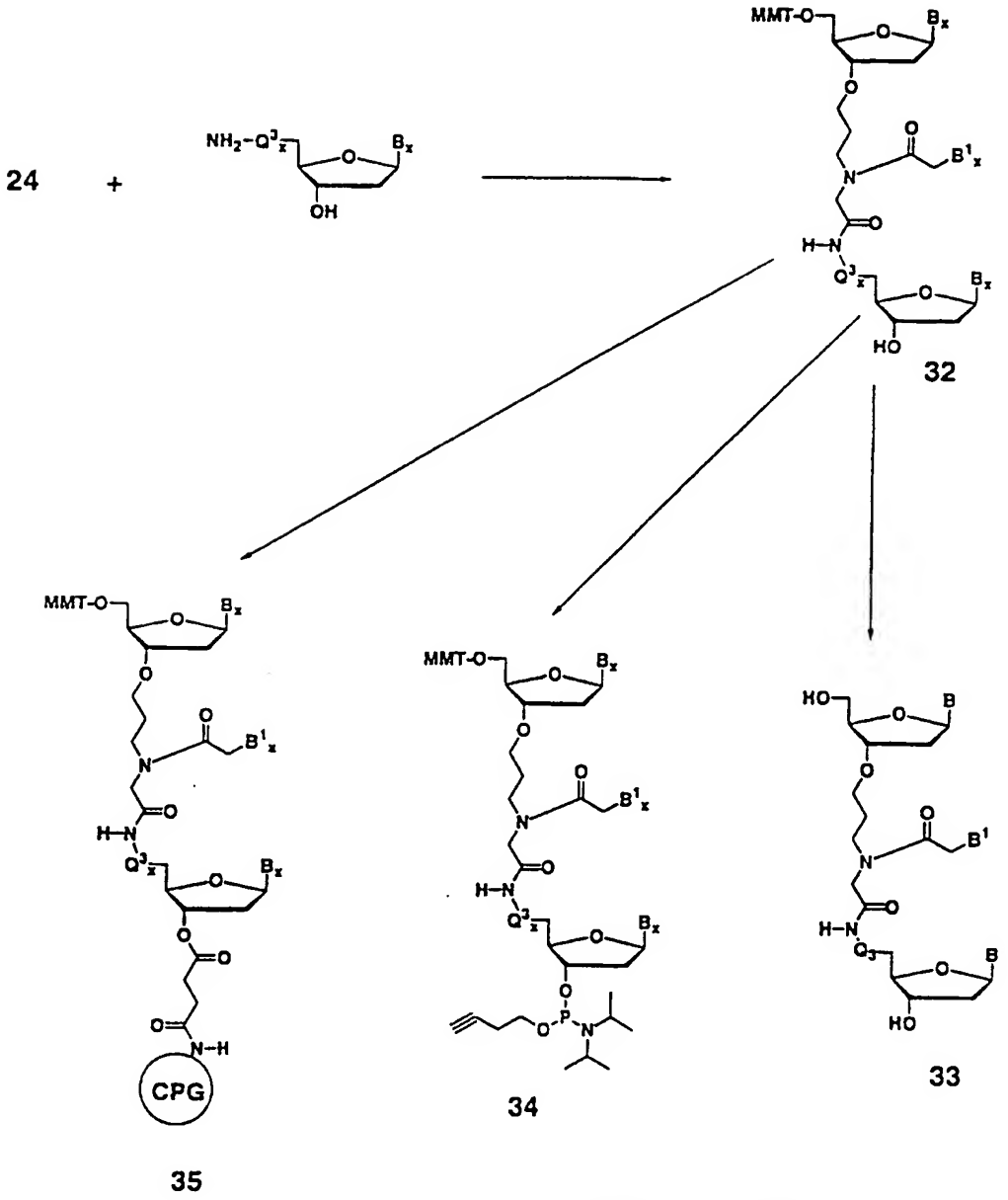


wherein B_x and B^1_x is selected independently from:
thymine-1-yl;
3-N-(benzyloxymethyl)thymine-1-yl;
4-N-(isobutyryl)cytidine-1-yl;
6-N-(phenoxyacetyl)adenine-9-yl;
2-N-(phenoxyacetyl)guanine-9-yl;
B and B^1 is selected independently from:
thymine-1-yl;
cytidine-1-yl;
adenine-9-yl;
guanine-9-yl.

Scheme III

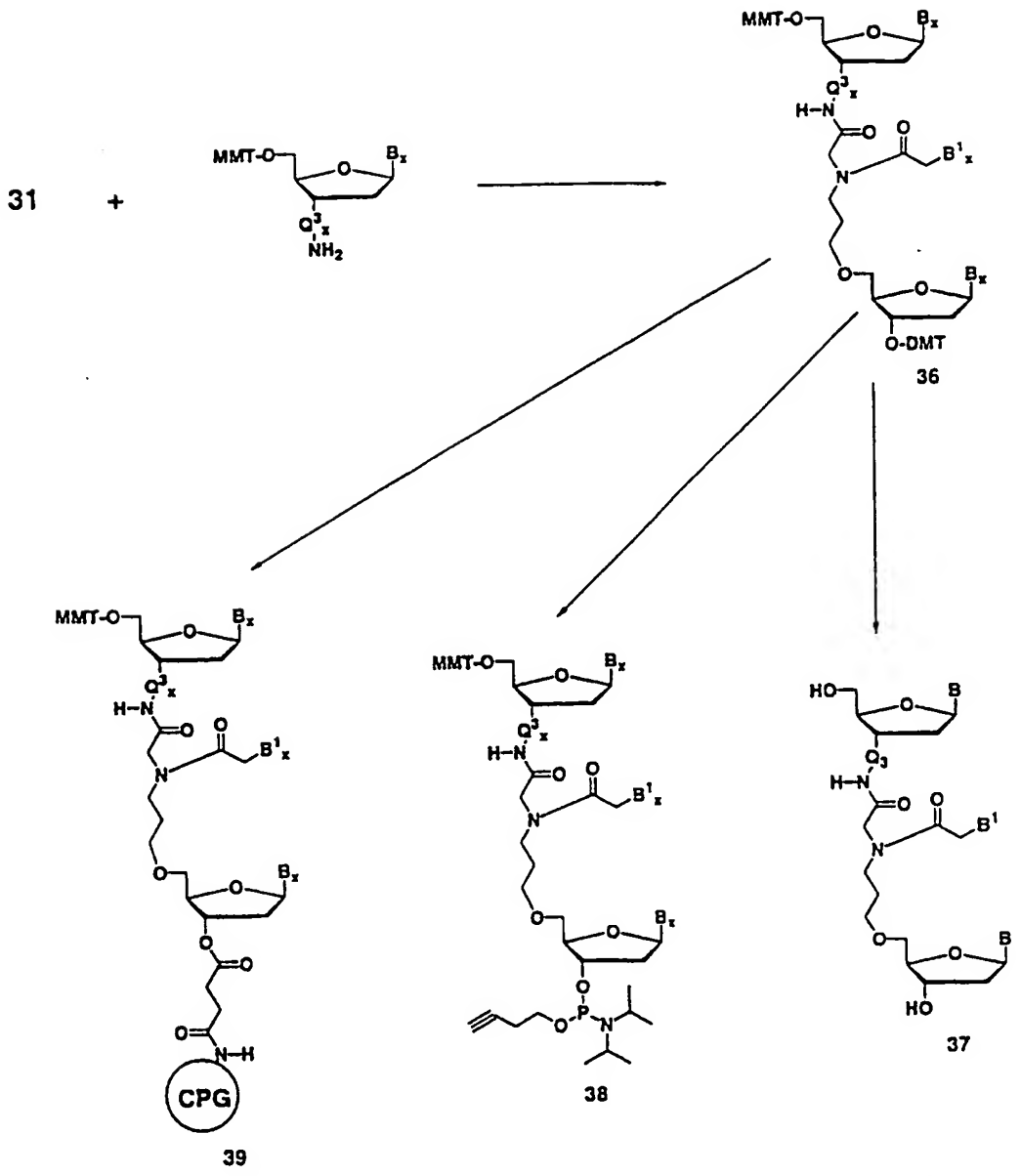

7
1. 60% NaH/DMF
2. CH2=CHCN
25
1. CoCl2/NaBH4/MeOH
2. CF3COOEt/EtOH
26
1. 60% NaH/DMF
2. BrCH2COO-t-Bu
27
1. H2/10%Pd/C
2. H+
28
1. NH3/EtOH
2. B'xCH2COOPfp
29
DMT-Cl/Py
30
1. HONSu/DCC
31

Scheme IV



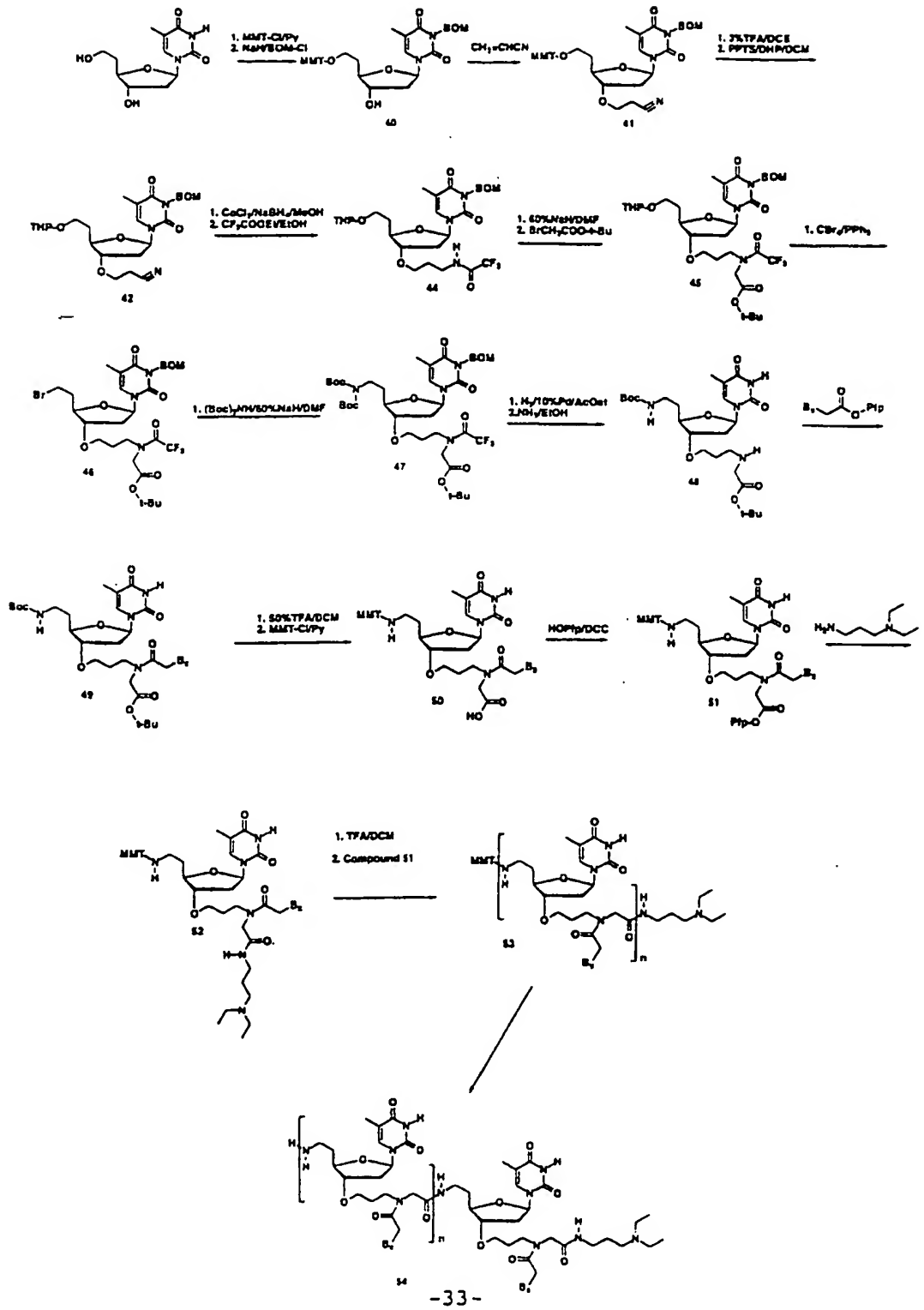
wherein $Q^3{}_x$ is selected from single bond, oxygen, -CH_2-, -N(CH_3)-, -N($COCF_3$)-, -N(Pac)-;
Q^3 is selected from single bond, oxygen, -CH_2-, -N(CH_3)-, -NH-.

Scheme V

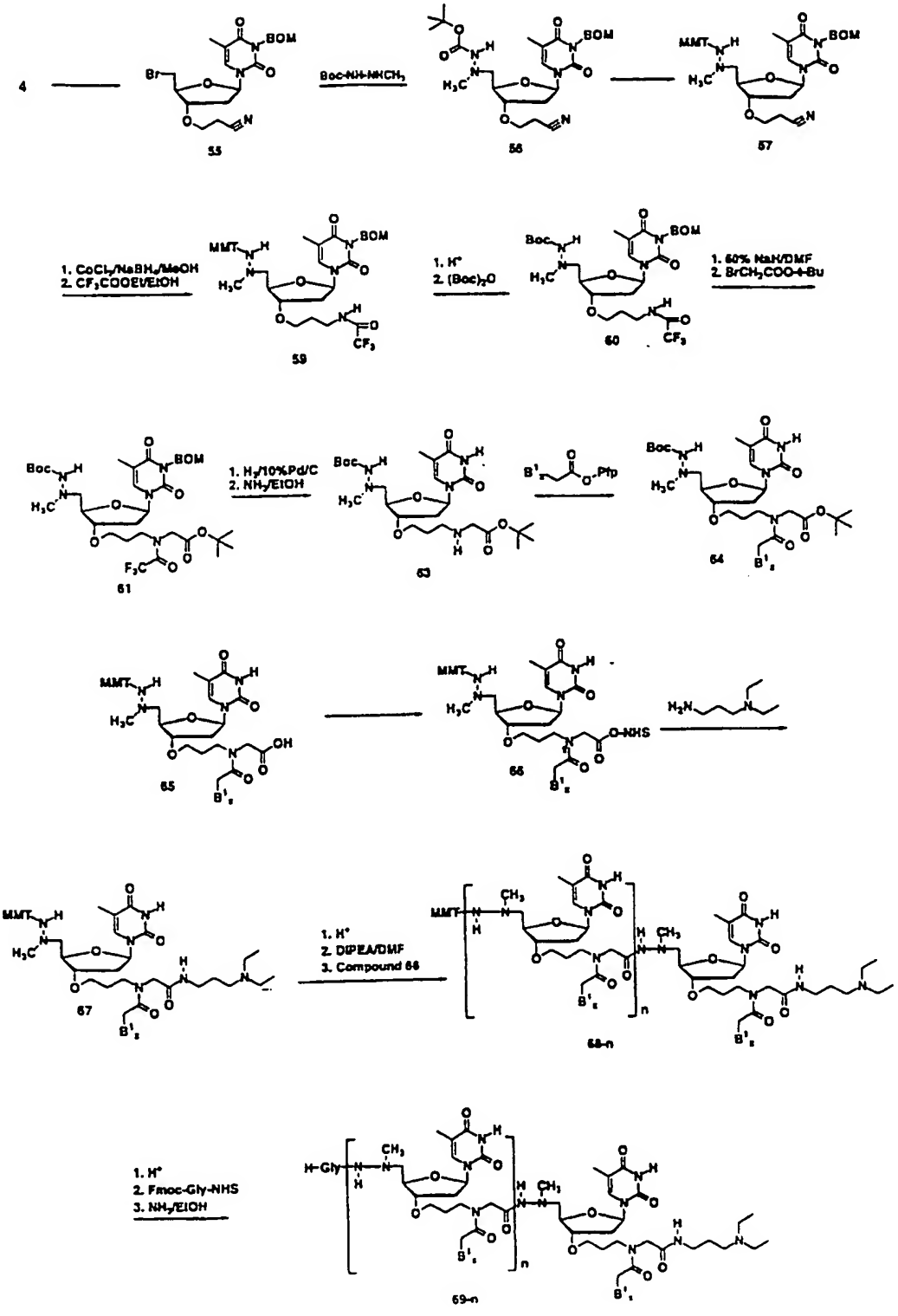


wherein Q^3_x is selected from single bond, oxygen, -CH_2-, -$N(CH_3)$-, -$N(COCF_3)$-, -N(Pac)-;
Q^3 is selected from single bond, oxygen, -CH_2-, -$N(CH_3)$-, -NH-.

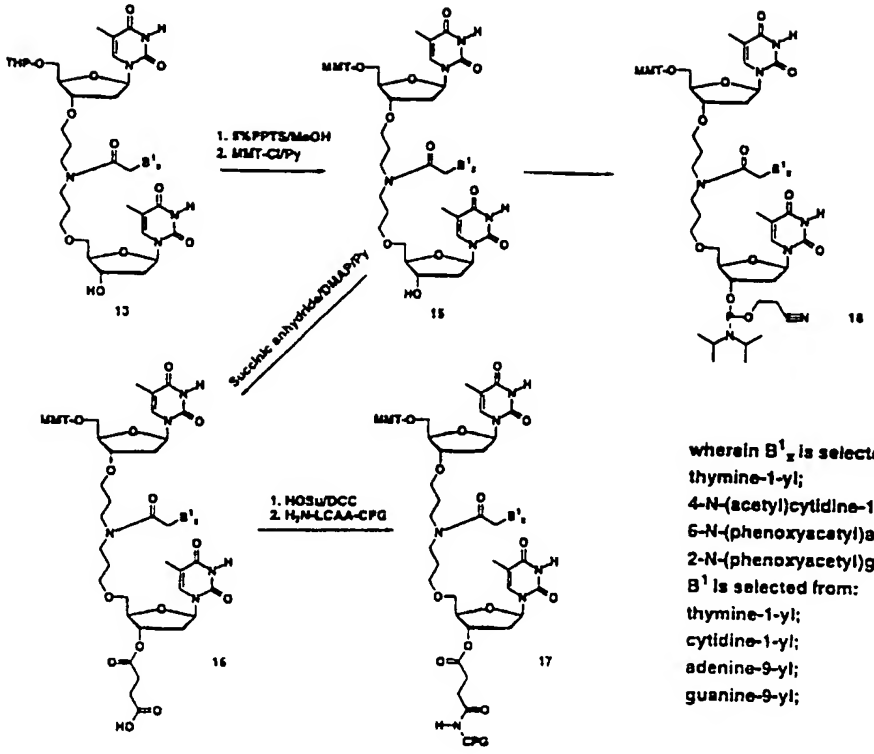
Scheme VI



Scheme VII

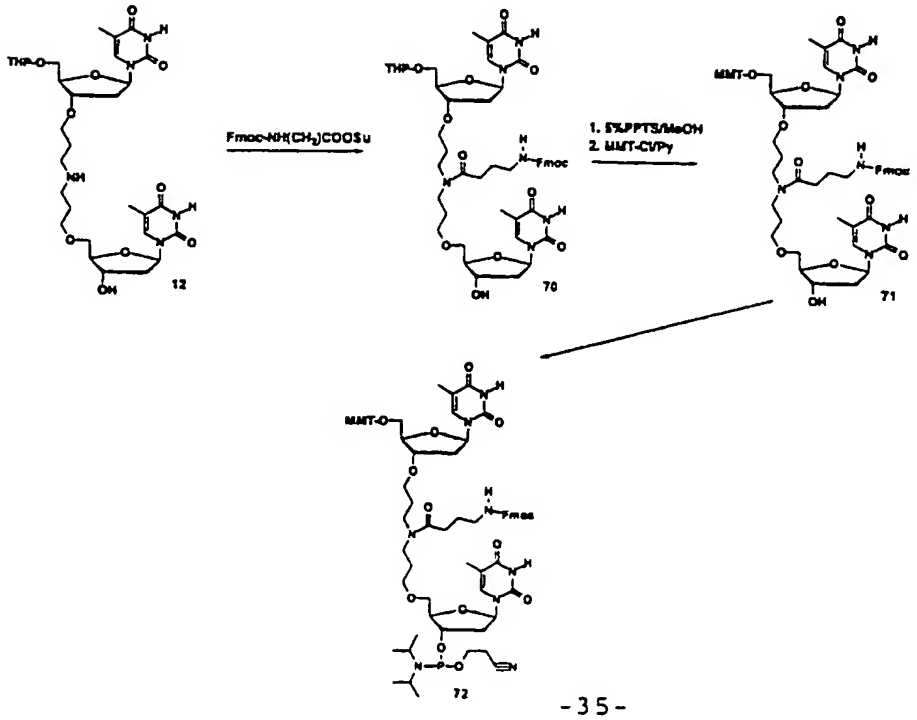

4
BOM
Br
55
Boc-NH-NHCH3
56
MMT
57
1. CoCl2/NaBH4/MeOH
2. CF3COOEt/EtOH
59
1. H+
2. (Boc)2O
60
1. 60% NaH/DMF
2. BrCH2COO-t-Bu
1. H2/10%Pd/C
2. NH2/EtOH
61
63
B'2
64
65
OH
66
O-NHS
67
1. H+
2. DIPEA/DMF
3. Compound 66
68-n
1. H+
2. Fmoc-Gly-NHS
3. NH2/EtOH
H-Gly
69-n

Scheme VIII

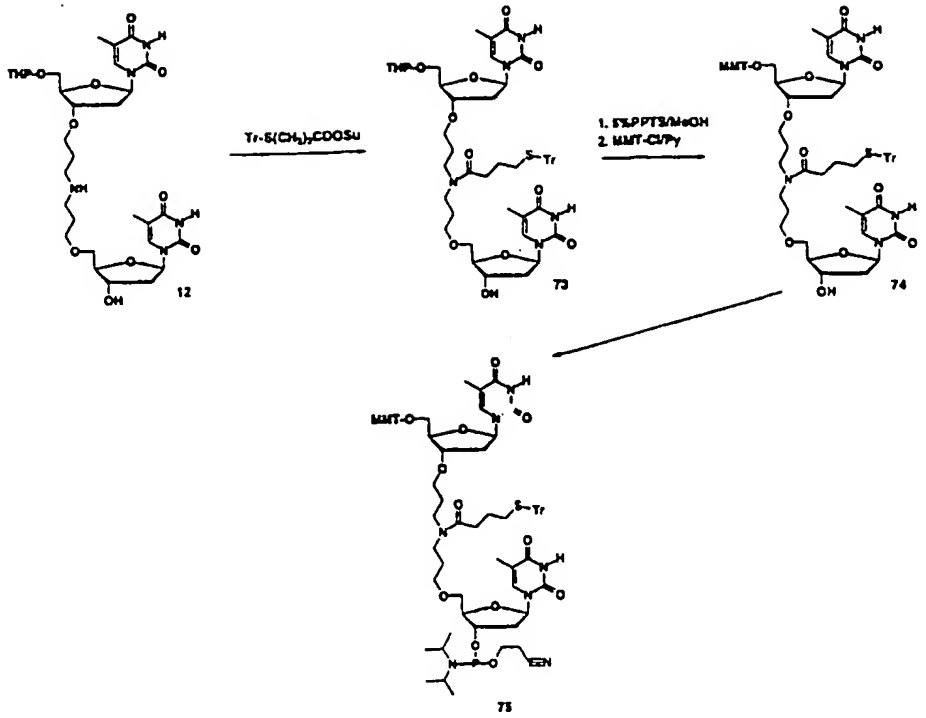


wherein B^1_x is selected from:
thymine-1-yl;
4-N-(acetyl)cytidine-1-yl;
6-N-(phenoxyacetyl)adenine-9-yl;
2-N-(phenoxyacetyl)guanine-9-yl;
B^1 is selected from:
thymine-1-yl;
cytidine-1-yl;
adenine-9-yl;
guanine-9-yl;

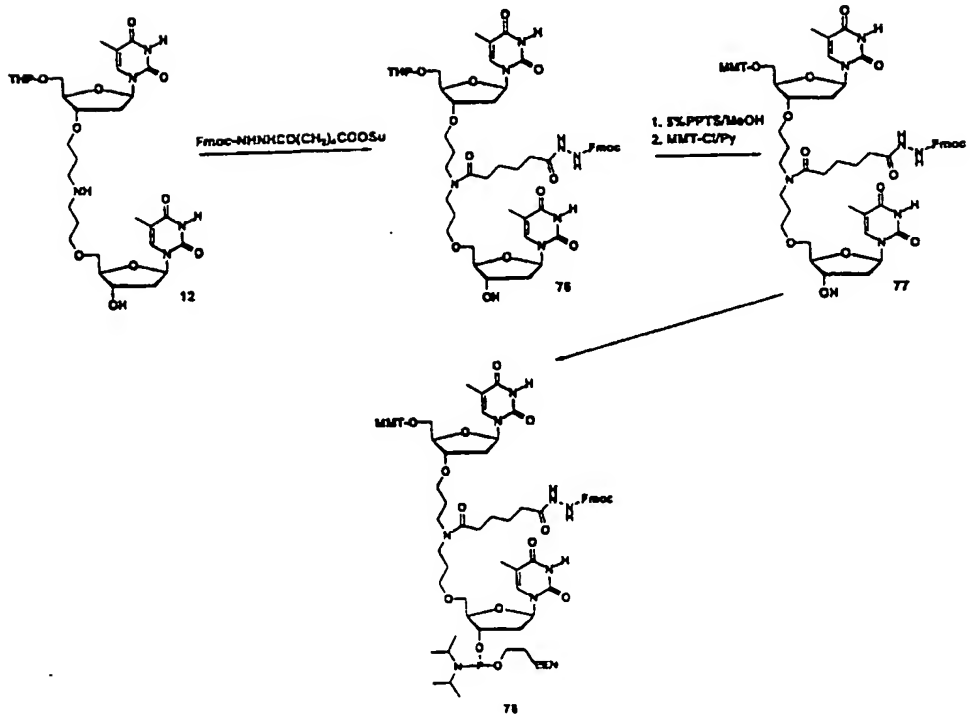
Scheme IX



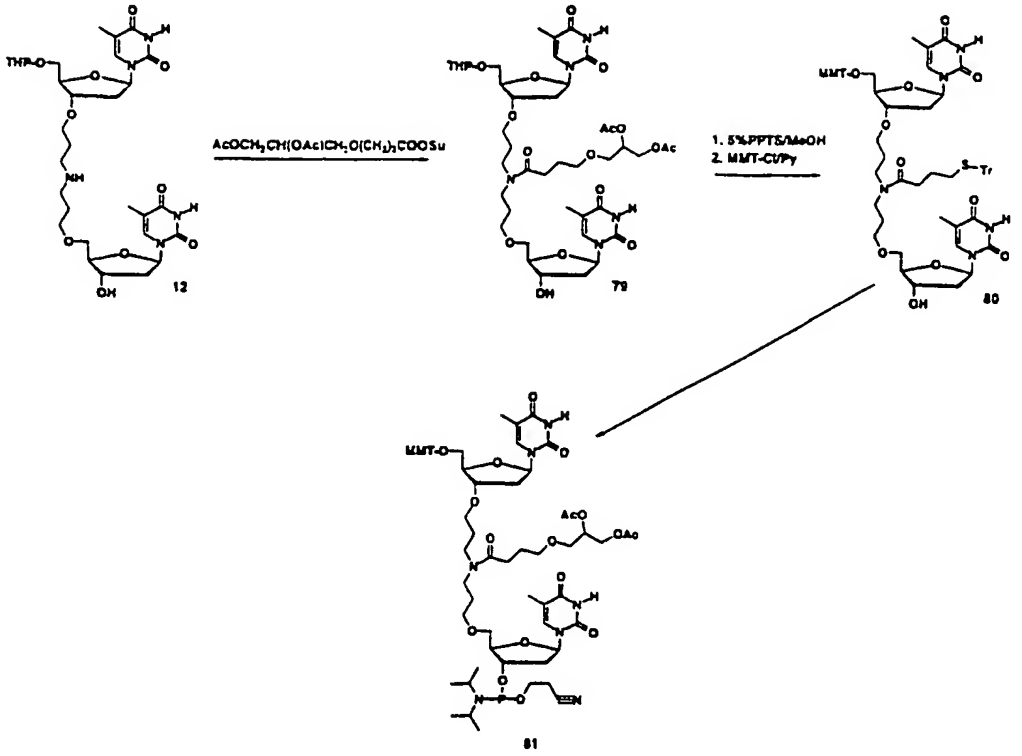
Scheme X



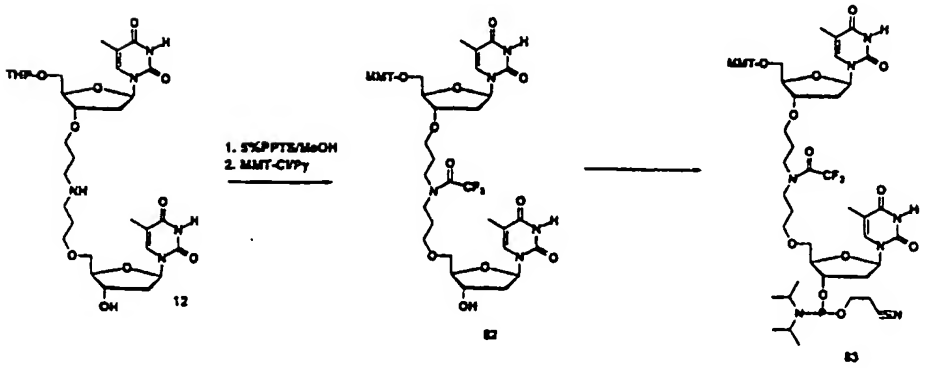
Scheme XI



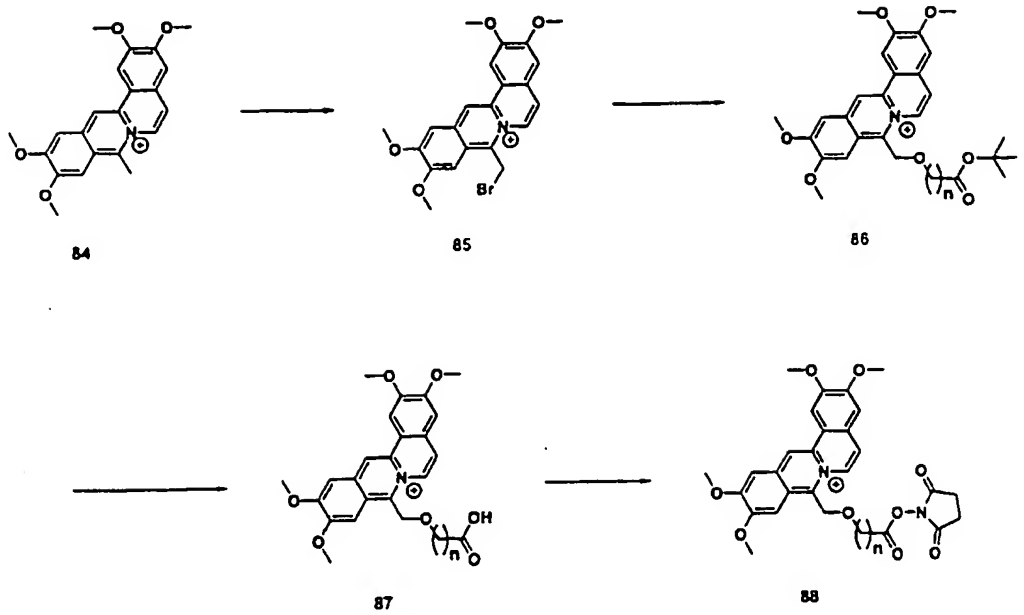
Scheme XII



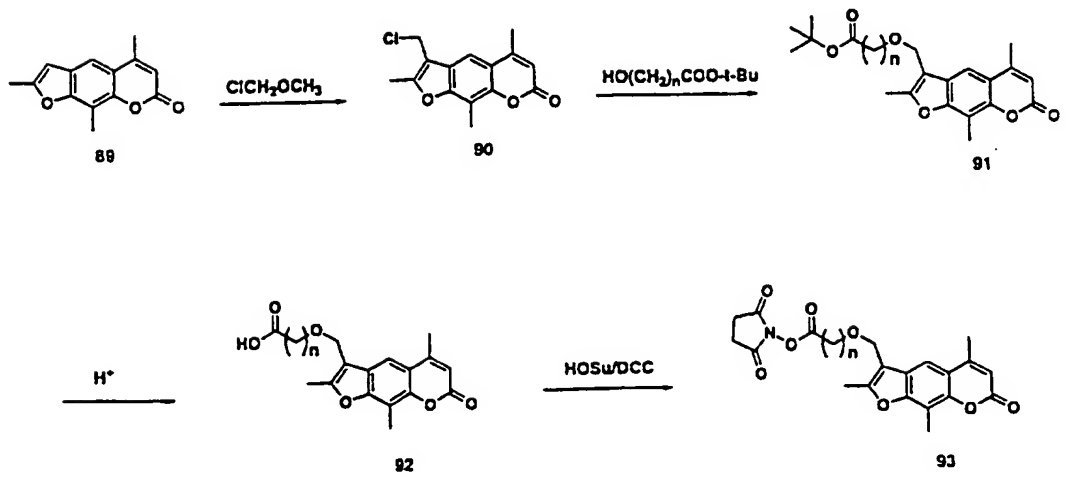
Scheme XIII



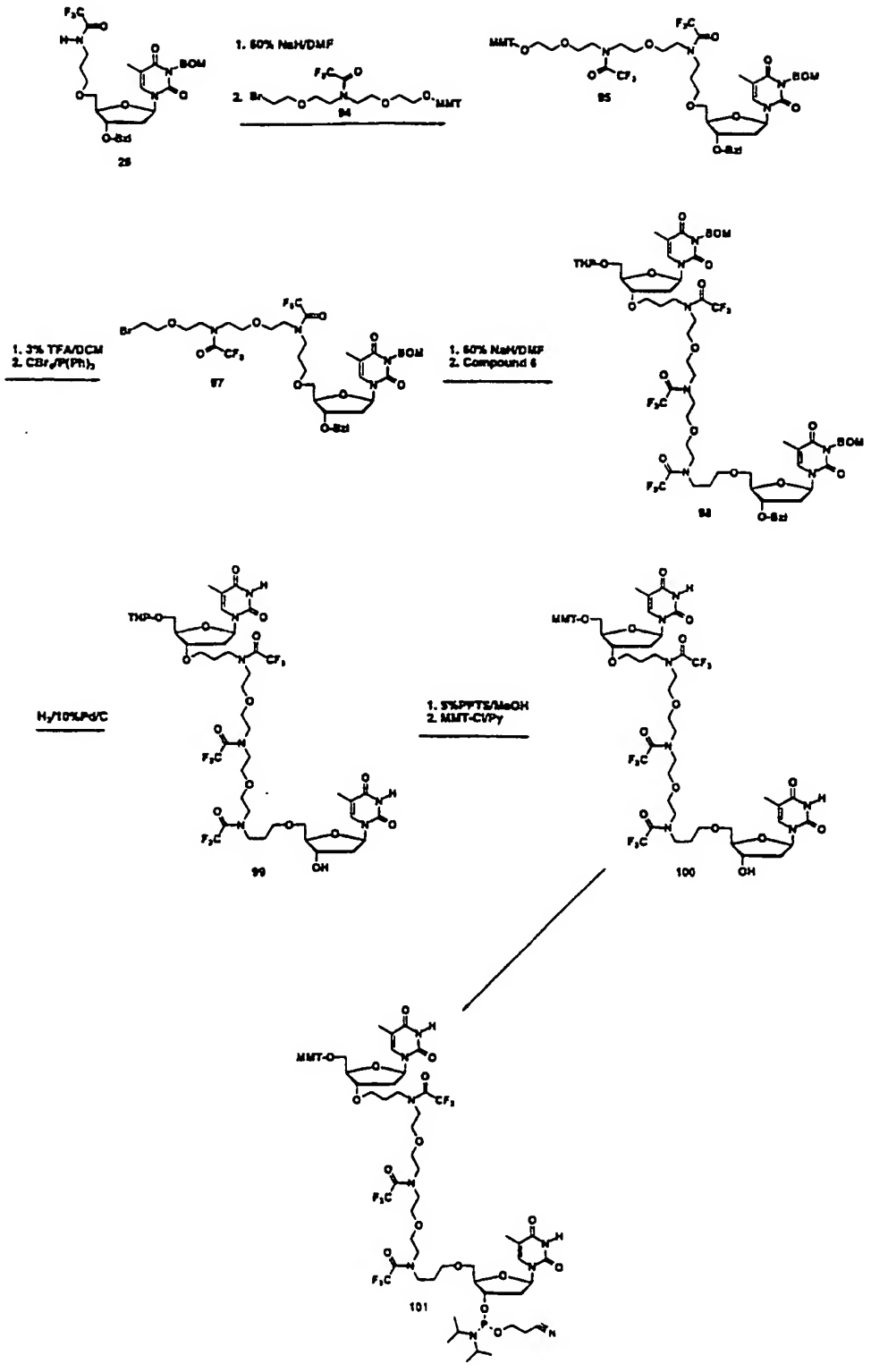
Scheme XIV



Scheme XV



Scheme XVI


1. 60% NaH/DMF
2.
1. 3% TFA/DCM
2. CBr4/P(Ph)3
1. 60% NaH/DMF
2. Compound 6
H2/10%Pd/C
1. 5%PPTS/MeOH
2. MMT-Cl/Py

Scheme I illustrates the synthesis of trinucleoside units containing fragments of formula B-1 (Fig. 1) with $-OCH_2CH_2CH_2N[X-B^1]-CH_2CH_2CH_2OCH_2-$ (3'→4') intersugar linkage. Nucleoside with a protected base and partially protected sugar moiety (compound **1**) is cyanoethylated (as in Example 2, *infra*) at the 3'-position, reduced to the aminopropyl derivative (compound **5**, Example 5) and then trifluoroacetylated (compound **6**, Example 6). The other chain of transformation is allylation of the 5'-position of the nucleoside followed by conversion to the 3-bromopropyl derivative (compound **9**). These reactions are exemplified in Examples 7, 8 and 9. Compound **6** is alkylated with compound **9** to yield compound **10**, that after deprotection of the bases and aza-nitrogen of the oligonucleosidic backbone gives compound **12** (Example 5). Compound **12** is a key substance for synthesis of various compounds of the present invention. For example, acylation of compound **12** with an activated ester of a carboxymethyl derivative of different heterocyclic bases (*e.g.*, thymine, cytosine, adenine and guanine) gives, respectively, trinucleosides **14a**, **14b**, **14c** and **14d** (as in Examples 16-18, *infra*). Acylation of compound **12** with an N-protected ω-amino acid derivative (*e.g.*, N-Fmoc-4-aminobutyric acid) and incorporation of this trimeric unit into an oligonucleotide chain gives, after deprotection, an oligonucleotide having an amino modified (abasic) site (motif E-2, Fig. 12) in its structure, wherein the aliphatic amino-group serves as a site of attachment for various DNA-active groups. Acylation of compound **12** with an S-protected ω-mercapto acid derivative (*e.g.*, 3-Tr-4-mercaptobutyric acid) and incorporation of this trimeric unit into an oligonucleotide chain gives, after deprotection, an oligonucleotide having a mercapto modified (abasic) site (motif E-3, Fig. 12) in its structure, wherein an aliphatic mercapto group serves as a site of attachment for various DNA-active groups. Acylation of compound **12** with an activated derivative of

2,3-di-O-Fmoc-1-O-carboxymethyl glycerine and incorporation of this trimeric unit into an oligonucleotide chain gives, after deprotection, an oligonucleotide having a *cis*-diol modified (abasic) site (motif E-4, Fig. 12) in its structure, wherein the *cis*-diol group after periodate oxidation provides an aldehyde group as a site of attachment for various DNA-binding groups. Acylation of compound **12** with an activated derivative of N-Fmoc-protected monohydrazido derivative of a dicarboxylic acid (*e.g.*, adipic, pimelic, suberic, *etc.*) and incorporation of this trimeric unit into an oligonucleotide chain gives, after deprotection, an oligonucleotide having a hydrazido modified (abasic) site (motif E-5, Fig. 12) in its structure, wherein the hydrazido group after deprotection serves as a site of attachment for various DNA-active groups.

Scheme II illustrates synthesis of intermediates for preparation of oligonucleosides containing fragments with a $-OCH_2CH_2CH_2N[X\text{-}B^1]CH_2C(=O)NHQ^3CH_2-$ (3′→4′) intersugar linkage. Such oligonucleotide analogs include compounds having formulas B-2, B-3, or B-4 (Fig. 1). Compound **6** (see Scheme I and Example 6) is a starting substance for the synthesis of such intermediates. Compound **6** is alkylated with the tert-butyl ester of bromoacetic acid in DMF in the presence of sodium hydride to give compound **19** (Example 23), that after hydrogenation is converted to compound **20** (Example 24). The trifluoroacetyl protecting group is removed from compound **20** by ammonolysis to give compound **21** (Example 25). Compound **21** is acylated with an activated carboxymethyl derivative of a nucleic base to give compound **22** (Examples 26, 27, 28), which after deprotection and the switching of the 5′-O-protecting group from tetrahydropyranyl to monomethoxytrytil is converted to the carboxylic acid derivative **23** (Examples 29, 30, 31). Compound **23** can be used for condensation directly, or transformed, for example, into activated ester **24** (Examples 32, 33, 34).

Scheme III illustrates synthesis of intermediates for preparation of oligonucleosides containing fragments with a $-Q^3NHC(=O)CH_2N[X\text{-}B^1]CH_2CH_2CH_2OCH_2-$ (3'→4') intersugar linkage. The oligonucleosides include compounds having formulas B-5, B-6, or B-7 (Fig. 2). Compound 7 (see Scheme I, Example 7) is a starting material for the synthesis of those intermediates. Compound 7 is cyanoethylated to give compound **25** (Example 35), that after reduction is trifluoroacetylated to compound **26** (Example 36). Compound **26** is alkylated with the tert-butyl ester of bromoacetic acid in DMF in the presence of sodium hydride to give compound **27** (Example 37), which after acidic deprotection is converted into a carboxylic acid derivative **28** (Example 38). Compound **28** is treated with a saturated solution of ammonia in ethanol and acylated with an activated carboxymethyl derivative of a nucleic base to give compound **29** (Examples 39, 40 and 41). Compound **29** is dimethoxytritylated to protect the 3'-hydroxyl group and to give compound **30** (Example 42), which can be used for condensation directly, or transformed, for example, into activated ester **31** (Example 43).

Scheme IV illustrates examples of synthesis of trinucleosides **33** containing motifs of formulas B-2, B-3, or B-4 (Fig. 1) starting from compounds of general formula **24**. Also, Scheme IV shows a route of conversion of partially protected trinucleoside **32** into phoshoramidite **34** and CPG derivative **35**.

Scheme V illustrates examples of synthesis of trinucleosides **37** containing motifs of formulas B-5, B-6, or B-7 (Fig. 2) starting from compounds of general formula **31**. Also Scheme V demonstrates the conversion of partially protected trinucleoside **36** into phoshoramidite **38** and CPG derivative **39**.

Scheme VI illustrates synthesis of peptide-like oligonucleosides **54** containing $-OCH_2CH_2CH_2N[X\text{-}B^1]CH_2C(=O)NHCH_2CH_2-$ (3'→4') intersugar

linkages. The methodology of synthesis is similar to that described in Scheme I. Homonucleoside with a protected base and partially protected sugar moiety (compound **40**, Example 43) is cyanoethylated at the 3'-position to give compound **41** (Example 44), which after switching of the 5'-O-protecting group from monomethoxytrytil to tetrahydropyranyl, is converted to compound **42** (Example 45). Compound **42** is reduced to the aminopropyl derivative (compound **43**, Example 46) and then amino group is blocked with trifluoroacetyl protecting group to give compound **44** (Example 47). Compound **44** is alkylated with the tert-butyl ester of bromoacetic acid in the presence of sodium hydride (compound **45**, Example 48). The 6'-O-THP group is converted to the bromo derivative (compound **46**, Example 49) through the treatment with $CBr_4/P(Ph)_3$ in THF. Compound **46** is treated with the sodium salt of di-tert-butyliminodicarboxylate in DMF to give compound **47** (Example 50), which after hydrgenolysis and ammonia treatment is converted to compound **48**. Compound **48** is acylated with an activated carboxymethyl derivative of a nucleic base to give modified dinucleoside **49** (Examples 52 and 53). Acid labile protecting groups (Boc- and *tert*-butyl protecting groups) are removed with 50% TFA/DCM and the 6'-amino function is blocked with a monomethoxytrytil protecting group to give compound **50** (Examples 54 and 55). Compound **50** can be used as a building block for solid-phase peptide-like synthesis of oligonucleosides with regular repeating elements depicted in square brackets in formula of compound **54**. Compound **50** also can be used as a building block for the synthesis of peptide-like oligonucleosides in solution as shown in Scheme VI (compounds **53** and **54**, Examples 59-64).

Scheme VII illustrates the synthesis of peptide-like oligonucleosides **69** containing $-OCH_2CH_2CH_2N[X-B^1]CH_2C(=O)NHN(CH_3)CH_2-$ (3'→4') intersugar linkages. The methodology of synthesis is similar to described in Scheme

VI.

Scheme VIII illustrates the synthesis of trinucleoside phoshoramidite **18** and trinucleoside CPG derivative **17**, containing the motif of formula B-1 (Fig. 1) starting from compounds of general formula **13** (see Scheme I).

Scheme IX illustrates the synthesis of 5'-monomethoxytrytil protected trinucleoside (compound **71**) with an abasic site containing an aminoalkyl linker, and conversion of that compound into phoshoramidite **72**. Incorporation of phoshoramidite **72** into an oligonucleotide chain gives, after deprotection, an oligonucleotide having an amino modified (abasic) site (motif E-2, Fig. 12) in its structure, wherein the aliphatic amino-group is utilized as a site of selective attachment for various DNA-active groups, *e.g.*, an acridine moiety (Structure C-1, Figure 7); a coralyne moiety (Structure C-8, Figure 9); or psoralen moiety (Structure D-1, Figure 10).

Scheme X illustrates the synthesis of 5'-monomethoxytrytil protected trinucleoside (compound **74**) with an abasic site containing an aliphatic mercapto group, and conversion of this compound into phoshoramidite **75**. Incorporation of phoshoramidite **75** into an oligonucleotide chain gives, after deprotection, an oligonucleotide having a mercapto modified (abasic) site (motif E-3, Fig. 12) in its structure, wherein the aliphatic amino-group is utilized as a site of selective attachment for various DNA-active groups, *e.g.*, a psoralen moiety (Structure D-2, Figure 10).

Scheme XI illustrates the synthesis of trinucleoside phoshoramidite **75** containing a Fmoc-protected hydrazido modified abasic site (motif E-5, Fig. 13).

Scheme XII illustrates the synthesis of trinucleoside phoshoramidite **81** with a 2,3-di-O-Ac-1-O-carboxymethyl glycerol modified abasic site (motif E-4, Fig. 12). Incorporation of that trimeric unit into an oligonucleotide chain gives, after deprotection, an

oligonucleotide having a *cis*-diol modified abasic site (motif E-4, Fig. 12) in its structure, wherein a *cis*-diol group, after periodate oxidation, provides an aldehyde group as a site of attachment for various DNA-active groups.

Scheme XIII illustrates the synthesis of phoshoramidite **83** with an abasic site containing a trifluoroacetyl protected aza-nitrogen in a trinucleoside backbone (motif E-1, Fig. 12). Incorporation of this trimeric unit into an oligonucleotide chain gives, after deprotection, an oligonucleotide with an abasic site, containing no substituents at the aza-nitrogen in the trinucleoside backbone (motif E-1, Fig. 12) in its structure.

Scheme XIV illustrates the synthesis of an activated ester of a carboxyl derivative of coralyne **88**. Compound **88** is used for postsynthetic modification of oligonucleotides containing an aminoalkyl linker with a coralyne moiety.

Scheme XV illustrates the synthesis of an activated ester of a carboxyl derivative of psoralen **93**. Compound **93** is used for postsynthetic modification of oligonucleotides containing an aminoalkyl linker with a psoralen moiety.

Scheme XVI illustrates the synthesis of phoshoramidite **101** with several abasic sites containing trifluoroacetyl protected aza-nitrogens in an oligonucleoside backbone (motif E-9, Fig. 14). Incorporation of that unit into an oligonucleotide chain gives, after deprotection, an oligonucleotide having regular phosphodiester oligonucleotide clusters connected with abasic polyoxaazaalkylene fragments containing no negatively charged phosphate groups in the fragment backbone (motif E-9, Fig. 14) in its structure.

The following Examples illustrate, but not limit, the compounds and utilities of the present invention.

All temperatures are in degrees Celsius (25°C refers

to ambient or room temperature). All parts not otherwise indicated are by weight, except for mixtures of liquids, which are by volume. The following abbreviations are employed: AcOEt for ethylacetate; DCE for 1,2-dichloroethane; DCM for dichloromethane; DIPEA for diisopropylethylamine; DMF for dimethylformamide; DMSO for dimethylsulfoxide; EtOH for ethanol; MeOH for methanol; THF for tetrahydrofuran; Py for pyridine; Phe for a phenyl radical; PPTS for pyridium p-toluenesulfonate; TFA for trifluoroacetic acid; Thy for a thymin-1-yl radical; Me for a methyl radical; Et for an ethyl radical; Gly for a glycine amino acid residue; dT for deoxythymidine; DMT for a dimethoxytrytil protecting group; MMT for a monomethoxytrytil (p-anisyldiphenylmethyl) protecting group; BOM for a benzyloxymethylene protecting group; CE for a cyanoethyl protecting group; Bzl for a benzyl protecting group; Boc for a tert-butoxycarbonyl protecting group; t-Bu for a tert-butyl protecting group; THP for a tetrahydropyranyl protecting group; NMR for nuclear magnetic resonance spectrum; MS for mass spectrum; TLC for thin layer chromatography on silica gel; HPLC for high pressure liquid chromatography; mp for melting point; mp d for melting point with decomposition; bp for boiling point. In reporting NMR data, chemical shifts are given in ppm and coupling constants (J) given in Hertz (Hz). All melting points are uncorrected.

Example 1

3-N-Benzyloxymethyl-5'-O-(p-anisyldiphenylmethyl) thymidine, 1

To a stirred solution of 5'-O-(p-anisyldiphenylmethyl) thymidine, (25.75 g, 50 mmole) in anhydrous DMF (350 ml) is added a suspension of 60% NaH (2.00 g, 50 mmole). The mixture is stirred at room temperature for 1 hour, and BOM-Cl (7.67 g, 49 mmole) is added. The mixture is stirred at room temperature for 2 hours, and DMF is evaporated. The residue is dissolved in

1 liter of a mixture AcOEt-H_2O. Organic layer is washed successively with water (2x250 ml) and brine (2x250 ml), dried ($MgSO_4$), evaporated *in vacuo* to dryness, and the residue is chromatographed over a silica gel column (8x35 cm) using toluene/AcOEt (gradient of AcOEt from 10 to 40%) as eluent to give 3-N-3'-O-(di-benzyloxymethyl)-5'-O-(p-anisyldiphenylmethyl)thymidine, 4.15 g (11%), as white foam, and compound 1, 27.00 (85%), as white foam. **^{1}H-NMR** ($CDCl_3$), 1: 7.59 (q, 1H, ^4J=1.0 Hz, H-6, Thy); 7.45-7.10 (gm, 17H, Phe, BOM&MMT); 6.82 (m, 2H, Phe, MMT); 6.42 (m, 1H, H-1', dT); 5.45 (s, 2H, NCH_2, BOM); 4.67 (s, 2H, OCH_2, BOM); 4.53 (m, 1H, H-3', dT); 4.05 (m, 1H, H-4', dT); 3.75 (s, OMe, MMT); 3.45 (m, 1H, H-5', dT); 3.32 (m, 1H, H-5', dT); 2.41 (m, 1H, H-2', dT); 2.25 (m, 1H, H-2', dT); 1.44 (d, 3H, CH_3, Thy). **^{13}C-NMR** ($CDCl_3$), 1: 163.5 (C-4, Thy); 158.6 (C-4, Phe-OMe, MMT); 150.9 (C-2, Thy); 143.8&143.6 (C-1, Phe, MMT); 137.7 (C-1, Phe, BOM); 134.7 (C-1, Phe-OMe, MMT); 134.5 (C-6, Thy); 130.3-125.2 (gs, C-2, C-3, C-4, Phe, BOM&MMT); 113.3 (C-3, Phe-OMe, MMT); 110.3 (C-5, Thy); 87.0 (-<u>C</u>(Phe)$_2$Phe-OMe); 86.0 (C-1', dT); 85.3 (C-4', dT); 72.1 (C-3', dT); 72.0 (OCH_2, BOM); 70.4 (NCH_2, BOM); 63.5 (C-5', dT); 55.1 (OMe, MMT); 41.0 (C-2', dT); 12.4 (CH_3, Thy).

Example 2

3-N-Benzyloxymethyl-3'-O-(2-cyanoethyl)-5'-O-(p-anisyldiphenylmethyl)thymidine, 2

To a stirred solution of compound 1 (12.70 g, 20 mmole) in anhydrous THF (150 ml) is added a suspension of 60% NaH (60 mg, 50 mmole) in one portion and dropwise neat acrylonitrile (5 ml) over 5 minutes. The resulting mixture is stirred at ambient temperature for an additional 1 hour. The mixture is evaporated *in vacuo* to dryness. The residue is dissolved in AcOEt (500 ml). The organic layer is

washed with brine (2x250 ml), dried ($MgSO_4$), evaporated *in vacuo* to dryness, and the residue is chromatographed over a silica gel column (4.5x30 cm) using toluene/AcOEt (gradient of AcOEt from 10 to 15%) as eluent to give compound 2, 12.66 g (92%), as a colorless oil. **^{1}H-NMR** ($CDCl_3$): 7.56 (q, 1H, ^4J=1.0 Hz, H-6, Thy); 7.45-7.15 (gm, 17H, Phe, BOM&MMT); 6.85 (m, 2H, Phe, MMT); 6.34 (m, 1H, $^3J_{1'-2'}$=5.7&8.0 Hz, H-1', dT); 5.49 (s, 2H, NCH_2, BOM); 4.71 (s, 2H, OCH_2, BOM); 4.19 (m, 1H, $^3J_{3'-4'}$=2.3 Hz, H-3', dT); 4.09 (m, 1H, $^3J_{4'-5'}$=2.7 Hz, H-4', dT); 3.79 (s, OMe, MMT); 3.90&3.76 (m&m, 1H+1H, H-5'); 3.60 (m, *, $O\underline{C}H_2CH_2CN$); 3.52 (m, 3H with *, H-5', dT); 3.34 (dd, 1H, $^2J_{5'-5'}$=10.6 Hz, H-5', dT); 2.54 (t, **, $OCH_2\underline{C}H_2CN$); 2.49 (m, 3H with **, $^3J_{2'-3'}$=2.2 Hz, $^2J_{2'-2'}$=13.6 Hz,H-2', dT); 2.20 (m, 1H, $^3J_{2'-3'}$=6.1 Hz, 1.52 (d, 3H, CH_3, Thy). **^{13}C-NMR** ($CDCl_3$): 163.4 (C-4, Thy); 158.8 (C-4, Phe-OMe, MMT); 150.9 (C-2, Thy); 143.7 (C-1, Phe, MMT); 137.9 (C-1, Phe, BOM); 134.6 (C-1, Phe-OMe, MMT); 134.1 (C-6, Thy); 130.3-127.3 (gs, C-2, C-3, C-4, Phe, BOM&MMT); 117.4 (-$\underline{C}$N); 113.3 (C-3, Phe-OMe, MMT); 110.5 (C-5, Thy); 87.2 (-$\underline{C}$(Phe)$_2$Phe^{1}OMe); 85.3 (C-1', dT); 83.7 (C-4', dT); 80.3 (C-3', dT); 72.1 (OCH_2, BOM); 70.4 (NCH_2, BOM); 63.9 ($O\underline{C}H_2CH_2CN$); 63.5 (C-5', dT); 55.2 (OMe, MMT); 37.6 (C-2', dT); 18.8 ($OCH_2\underline{C}H_2CN$); 12.5 (CH_3, Thy).

Example 3

3-N-Benzyloxymethyl-3'-O-(2-cyanoethyl)thymidine, 3

A solution of compound 2 (12.04 g, 17.5 mmole) in 3% TFA/DCE is kept at ambient temperature for 15 minutes. The reaction mixture is washed successively with 5% $NaHCO_3$ (3x150 ml) and water (2x150 ml), dried ($MgSO_4$), evaporated *in vacuo* to dryness and the residue is chromatographed over

a silica gel column (4.5x20 cm) using 3% MeOH/$CHCl_3$ as eluent to give compound 3, 6.90 g (95%), as a colorless oil. **MS**: [M+1]=416; [M+1+NH_3]=433. **^{1}H-NMR** ($CDCl_3$): 7.43 (s, 1H, H-6, Thy); 7.4-7.2 (gm, 5H, Phe, BOM); 6.12 (m, 1H, H-1'); 5.48 (s, 2H, NCH_2, BOM); 4.69 (s, 2H, OCH_2, BOM); 4.24 (m, 1H, H-3'); 4.09 (m, 1H, H-4'); 3.90&3.76 (m&m, 1H+1H, H-5'); 3.66 (m, 2H, $O\underline{CH_2}CH_2CN$); 2.97 (t, 1H, 5'-OH); 2.61 (t, 2H, $OCH_2\underline{CH_2}CN$); 2.34 (m, 2H, H-2'); 1.91 (s, 3H, CH_3, Thy). **^{13}C-NMR** ($CDCl_3$): 163.4 (C-4, Thy); 150.9 (C-2, Thy); 137.8 (C-1, Phe, BOM); 135.6(C-6, Thy); 128.19& 127.59&127.56(C-2, C-3, C-4, Phe, BOM); 110.2 (C-5, Thy); 85.4 (C-1', dT); 84.8 (C-4', dT); 79.7 (C-3', dT); 72.1 (OCH_2, BOM); 70.4 (NCH_2, BOM); 63.9 ($O\underline{CH_2}CH_2CN$); 62.4 (C-5', dT); 36.9 (C-2', dT); 19.0 ($OCH_2\underline{CH_2}CN$); 13.1 (CH_3, Thy).

Example 4

3-N-Benzyloxymethyl-3'-O-(2-cyanoethyl)-5'-O-(tetrahydropyran-2-yl)thymidine, 4

To a stirred solution of compound 3 (6.90 g, 16.6 mmole) in anhydrous DCM (150 ml) is added PPTS (100 mg, 0.4 mmole) and dropwise DHP (5 ml) over 15 minutes. The resulting mixture is stirred at ambient temperature for an additional 3 hours. The reaction mixture is evaporated *in vacuo* to dryness, coevaporated with toluene (2x100 ml), dissolved in AcOEt (250 ml) and washed successively with 5% $NaHCO_3$ (3x100 ml) and brine (2x100 ml). The residue is chromatographed over a silica gel column (4.5x30 cm) using toluene/AcOEt (1/1) as eluent to give compound 4, 7.62 g (92%), as a colorless oil. **MS**: [M+1]=500; [M+1+NH_3]=517. **^{1}H-NMR** ($CDCl_3$): 7.59&7.52(m&m,1H, H-6, Thy); 7.40-7.20 (gm, 5 H, Phe ,BOM); 6.31 (m, 1H, H-1'); 5.49 (s, 2H, $N\underline{CH_2}$, BOM); 4.69 (s, 2H, $O\underline{CH_2}$, BOM); 4.61 (m, 1H, H-1, THP); 4.22 (m, 2H with H-3', H-4'); 4.20 (m, 2H with H-4', H-3');

4.10-3.50 (gm, 6H, H-5' + H-5(THP) + OCH_2CH_2CN); 2.62 (t, 2H, OCH_2CH_2CN); 2.45 (m, 1H, H-2'); 2.05 (m, 1H, H-2'); 1.94&1.91(s&s, 3H, CH_3, Thy); 1.85-1.40 (gm, 6H, H-2,-3,-4, THP). ^{13}C-NMR ($CDCl_3$): 163.2 (C-4, Thy); 150.7 (C-2, Thy); 137.8 (C-1, Phe, BOM); 134.3&134.0(C-6, Thy); 128.1&127.5&127.4(C-2, C-3, C-4, Phe, BOM); 109.91&109.85 (C-5, Thy); 99.9&99.0 (C-1, THP); 85.7&85.6 (C-1', dT); 83.5&83.3 (C-4', dT); 80.5&80.4 (C-3', dT); 71.9 (OCH_2, BOM); 70.3 (NCH_2, BOM); 67.5&67.2 (C-5', dT); 63.83&63.77 (OCH_2CH_2CN); 63.3&62.8 (C-5, THP); 37.6&37.5 (C-2', dT); 30.6&30.5 (C-2, THP); 25.02&24.97 (C-4, THP); 20.0&19.7 (C-3, THP); 18.8 (OCH_2CH_2CN); 13.1&12.9 (CH_3, Thy).

Example 5

3-N-Benzyloxymethyl-3'-O-(3-aminopropyl)-5'-O-(tetrahydropyran-2-yl)thymidine, 5

To a stirred solution of compound 4 (7.0 g, 14 mmole) and $CoCl_2 \cdot 6H_2O$ (6.66 g, 28 mmole) in MeOH (150 ml) is added $NaBH_4$ (4.48 g, 140 mmole) in four portions over 15 minutes. The resulting mixture is stirred at ambient temperature for an additional 1 hour and 28% NH_4OH (70 ml) is added and stirred at ambient temperature for 15 minutes. The mixture is centrifuged, the supernatant is kept and the precipitate is washed twice with MeOH. The combined supernatants are evaporated, the residue is suspended in $CHCl_3$, the insoluble material is filtered off and the filtrate is evaporated *in vacuo* to dryness. The residue is chromatographed over a silica gel column (4.5x25 cm) using $CHCl_3$/MeOH/28%NH_4OH (100/3/0.25) as eluent to give compound 5, 4.37 g (62%), as a yellowish oil. MS: [M+1]=504. ^{1}H-NMR ($CDCl_3$): 7.61&7.54 (q&q,1H, H-6, Thy); 7.45-7.20 (gm, 5 H, Phe ,BOM); 6.32 (m, 1H, H-1', dT); 5.49 (s, 2H, NCH_2, BOM); 4.70 (s, 2H, OCH_2, BOM); 4.65&4.58 (m&m, 1H, H-1,

THP); 4.20 (m, 1H, H-4', dT); 4.09 (m, *, H-3', dT); 4.08&3.95&3.69&3.57 (dd&dd&dd&m, *, H-5', dT); 3.90&3.56 (m&m, *, H-5, THP); 3.50 (m, 7H with *, $O\underline{C}H_2CH_2CH_2NH_2$); 2.81 (t, 2H, $OCH_2CH_2\underline{C}H_2NH_2$); 2.44 (m, 1H, H-2', dT); 2.00 (m, **, H-2', dT); 1.94&1.91(d&d, **, CH_3, Thy); 1.72 (m, **, $OCH_2CH_2\underline{C}H_2NH_2$); 1.78&1.57 (m&m, 12H with **, H-2, -3,-4, THP); 1.38 (bs, 2H, $OCH_2CH_2CH_2\underline{N}H_2$). **^{13}C-NMR** ($CDCl_3$): 163.4 (C-4, Thy); 150.8 (C-2, Thy); 137.9 (C-1, Phe, BOM); 134.4&134.2(C-6, Thy); 128.1&127.6&127.5 (C-2, C-3, C-4, Phe, BOM); 109.9 (C-5, Thy); 99.9&99.8 (C-1, THP); 85.9&85.8 (C-1', dT); 83.8&83.7 (C-4', dT); 79.9&79.8 (C-3', dT); 72.0 (OCH_2, BOM); 70.3 (NCH_2, BOM); 67.9&67.4 (C-5', dT); 67.3&67.2 ($O\underline{C}H_2CH_2CH_2NH_2$); 63.2&62.5 (C-5, THP); 39.2 ($OCH_2CH_2\underline{C}H_2NH_2$); 37.9&37.8 (C-2', dT); 33.29&33.26 ($OCH_2\underline{C}H_2CH_2NH_2$); 30.60&30.55 (C-2, THP); 25.12&25.09 (C-4, THP); 20.0&19.5 (C-3, THP); 13.1&13.0 (CH_3, Thy).

Example 6

3-N-Benzyloxymethyl-3'-O-(3-trifluoroacetamidopropyl)-5'-O-(tetrahydropyran-2-yl)thymidine, 6

A solution of compound 5 (4.00 g, 8.0 mmole), DIPEA (0.5 ml) and CF_3COOEt (7 ml) in anhydrous EtOH is kept at ambient temperature overnight. The reaction mixture is evaporated *in vacuo* to dryness and the residue is chromatographed over a silica gel column (4.5x25 cm) using toluene/AcOEt (7/3) as eluent to give compound 6, 4.46 g (93%), as a colorless oil. **MS**: [M+1]=600; [M+1+NH_3]=617. **^{1}H-NMR** ($CDCl_3$): 7.60&7.53(q&q, 1H, H-6, Thy); 7.40-7.20 (gm, *, Phe , BOM); 7.30 (m, 6H with *, $\underline{NH}COCF_3$); 6.29 (m, 1H, H-1', dT); 5.49 (s, 2H, $N\underline{C}H_2$, BOM); 4.69 (s, 2H, $O\underline{C}H_2$, BOM); 4.65&4.58 (m&m, 1H, H-1, THP); 4.17 (m, **, H-4', dT); 4.14&4.09 (m&m, **, H-3', dT); 4.07&3.96&3.71&3.57

(dd&dd&dd&m, **, H-5', dT); 3.90&3.56 (m&m, **, H-5, THP); 3.58 (m, **, $O\underline{C}H_2CH_2CH_2NHCOCF_3$); 3.50 (m, 10H with **, $OCH_2CH_2\underline{C}H_2NHCOCF_3$); 2.43 (m, 1H, H-2', dT); 2.03 (m, ***, H-2', dT); 1.95&1.91(d&d, ***, CH_3, Thy); 1.88 (m, ***, $OCH_2\underline{C}H_2CH_2NHCOCF_3$); 1.57&1.37 (m&m, 12H with ***, H-2,-3,-4, THP). **^{13}C-NMR** ($CDCl_3$): 163.4&163.3 (C-4, Thy); 157.0 (q, $^2J_{C-F}$=61.7 Hz, $\underline{C}(=O)CF_3$); 150.8 (C-2, Thy); 137.8 (C-1, Phe, BOM); 134.4&134.1 (C-6, Thy); 128.1&127.5 (C-2, C-3, C-4, Phe, BOM); 115.8 (q, $^1J_{C-F}$=287.4 Hz, $C(=O)\underline{C}F_3$); 110.0&109.9 (C-5, Thy); 100.0&99.1 (C-1, THP); 85.8&85.7 (C-1', dT); 83.7&83.6 (C-4', dT); 80.4&80.3 (C-3', dT); 72.0 (OCH_2, BOM); 70.3 (NCH_2, BOM); 67.9&67.8 ($O\underline{C}H_2CH_2CH_2NHCOCF_3$); 67.3&67.2 (C-5', dT); 63.3&62.9 (C-5, THP); 38.4 ($OCH_2CH_2\underline{C}H_2NHCOCF_3$); 37.6&37.5 (C-2', dT); 30.59&30.57 (C-2, THP); 28.3 ($OCH_2\underline{C}H_2CH_2NHCOCF_3$); 25.1&25.0 (C-4, THP); 20.1&19.7 (C-3, THP); 13.1&12.9 (CH_3, Thy). **^{19}F-NMR** ($CDCl_3$): -76.23 (s, $C\underline{F}_3$).

Example 7

3-N-Benzyloxymethyl-3'-O-(benzyl)thymidine, 7

To a stirred solution of compound **1** (33.25 g, 50 mmole) in anhydrous DMF (150 ml) is added a suspension of 60% NaH.(2.4 g, 60 mmole) and after 1 hour dropwise benzyl bromide (12.0 g, 8.4 ml, 70 mmole) over a period of 5 minutes. The resulting mixture is stirred at ambient temperature overnight, and the DMF is evaporated *in vacuo* to dryness. The residue is dissolved in AcOEt (600 ml), washed successively with water (1x200 ml) and brine (2x200 ml), dried ($MgSO_4$) and evaporated *in vacuo* to dryness. The residue is coevaporated with toluene (2x300), dissolved in 3% TFA/DCE (700 ml) and kept at ambient temperature for 15 minutes. The reaction mixture is washed successively with 5% $NaHCO_3$ (3x250 ml) and water (2x250 ml), dried ($MgSO_4$)

and evaporated *in vacuo* to dryness. The residue is crystallized from toluene/hexane to give compound 7, 17.42 g (77%), as white crystals. Another 2.15 g (9.5%) of compound 7 can be isolated by chromatography of mother liquor over silica gel column (2.5x25 cm) using toluene/AcOEt (7/3) as eluent. **^{1}H-NMR** ($CDCl_3$): 7.42 (q, 1H, ^4J=0.9 Hz, H-6, Thy); 7.45-7.17 (gm, 10H, Phe ,BOM&Bzl); 6.16 (m, 1H, $^3J_{1',-2'}$=6.3&7.6 Hz, H-1', dT); 5.48 (s, 2H, $N\underline{CH_2}$, BOM); 4.69 (s, 2H, $O\underline{CH_2}$, BOM); 4.57&4.50 (d&d, 2H, ^2J=11.6 Hz, OCH_2, Bzl); 4.26 (m, 1H, $^3J_{3',-4'}$=2.9 Hz, $^3J_{3',-2'}$=2.9&6.4 Hz,H-3',dT); 4.15 (m, $^3J_{5',-4'}$ =2.8&2.9 Hz, H-4', dT); 3.90&3.74 (dd&dd, 2H, $^2J_{5',-5'}$=11.8 Hz, H-5', dT); 2.42 (m, 1H, $^2J_{2',-2'}$=13.6 Hz, H-2', dT); 2.03 (m, 1H, H-2', dT); 1.90(d, 3H, CH_3, Thy). **^{13}C-NMR** ($CDCl_3$): 163.4 (C-4, Thy);150.9 (C-2, Thy); 137.8 (C-1, Phe, BOM); 137.4 (C-1, Phe, Bzl), 135.6 (C-6, Thy); 128.5&128.2&127.9&127.6 (C-2, C-3, C-4, Phe, BOM&Bzl); 110.2 (C-5, Thy); 87.6 (C-1', dT); 85.0 (C-4', dT); 78.6 (C-3', dT); 72.1 (OCH_2, BOM); 71.5 (OCH_2, Bzl); 70.3 (NCH_2, BOM); 62.8 (C-5', dT); 37.2 (C-2', dT); 13.2 (CH_3, Thy).

Example 8

3-N-Benzyloxymethyl-3'-O-(benzyl)-5'-O-(allyl)thymidine, 8

To a stirred solution of compound 7 (6.75 g, 15 mmole) in anhydrous DMF (75 ml) is added a suspension of 60% NaH (0.80 g, 20 mmole) and after 1 hour dropwise allyl bromide (3.02 g, 2.16 ml, 25 mmole) over 5 minutes. The resulting mixture is stirred at ambient temperature for 2 hours, and the DMF is evaporated *in vacuo* to dryness. The residue is dissolved in AcOEt (300 ml), washed successively with water (1x100 ml) and brine (2x100 ml), dried ($MgSO_4$) and evaporated *in vacuo* to dryness. The residue is

chromatographed over silica gel column (2.5x25 cm) using toluene/AcOEt (9/1) as eluent to give compound 8, 7.05 g (95%), as a colorless oil. **MS**: [M+1]=493; [M+1+NH_3]=510. **^{1}H-NMR** ($CDCl_3$): 7.60 (q, 1H, ^4J=1.0 Hz, H-6, Thy); 7.40-7.15 (gm, 10H, Phe ,BOM&Bzl); 6.40 (m, 1H, $^3J_{1'-2'}$=6.0&7.5 Hz, H-1', dT); 5.88 (m, 1H, $^3J_{CH2=CH}$=10.2&17.4 Hz, $^3J_{CH-CH2}$=5.4 Hz, CH_2=C<u>H</u>-CH_2-); 5.48 (s, 2H, N<u>CH_2</u>, BOM); 5.24 (m, 2H, <u>CH_2</u>=CH-CH_2-); 4.69 (s, 2H, O<u>CH_2</u>, BOM); 4.57&4.48 (d&d, 2H, ^2J=11.9 Hz, OCH_2, Bzl); 4.24 (gm, 2H,, H-3', -4', dT); 4.01 (bd, 2H, CH_2=CH-<u>CH_2</u>-); 3.72&3.56 (dd&dd, 2H, $^2J_{5'-5'}$=10.2, Hz, H-5', $^3J_{5'-4'}$ =2.2 Hz, dT); 2.47 (m, 1H, $^2J_{2'-2'}$=12.2 Hz, $^3J_{3'-2'}$=2.4 Hz H-2'; dT); 2.03 (m, 1H, $^3J_{3'-2'}$=5.8 Hz, H-2', dT); 1.89 (d, 3H, CH_3, Thy). **^{13}C-NMR** ($CDCl_3$): 163.3 (C-4, Thy);150.8 (C-2, Thy); 137.9 (C-1, Phe, BOM); 137.3 (C-1, Phe, Bzl), 134.5 (C-6, Thy); 133.8 (CH_2=<u>C</u>H-CH_2-); 128.3&128.0&127.9&127.7&127.5&127,4&127,3 (C-2, C-3, C-4, Phe, BOM&Bzl); 117.2 (<u>CH_2</u>=CH-CH_2-); 119.8 (C-5, Thy); 87.6 (C-1', dT); 83.7 (C-4', dT); 79.0 (C-3', dT); 72.1 (CH_2=CH-<u>CH_2</u>-); 71.9 (OCH_2, BOM); 71.1 (OCH_2,, Bzl); 70.2 (NCH_2, BOM); 70.1 (C-5', dT); 63.3&62.9 (C-5, THP); 37.8 (C-2', dT); 13.1 (CH_3, Thy).

Example 9

3-N-Benzyloxymethyl-3'-O-(benzyl)-5'-O-(3-bromopropyl) thymidine, 9

A dry 50-ml flask equiped with septum inlet, thermometer well, and magnetic stirrer is flashed with argon and then maintained under positive argon pressure. The flask is charged with a solution of compound 8 (4.5 g, 9.1 mmole) in anhydrous THF (10 ml) and cooled to 0°C. A 1M solution of BH_3/THF (3.1 ml, 9.3 mmole) is added

dropwise over a period of 5 minutes. The resulting mixture is stirred for 30 min at 0°C and 30 min at ambient temperature. Then MeOH (60 µl) is added to destroy excess of hydride. The solution is cooled to -10°C and bromine (1.94 g, 0.62 ml, 12.1 mmole) is added dropwise over 10 minutes. Then a 4.34 M solution of NaOMe (3.5 ml, 15.2 mmole) is added dropwise over 45 minutes at 0°C. The reaction mixture is diluted with AcOEt (150 ml) and washed successively with saturated $NaHCO_3$ (3x50 ml) and brine (2x50 ml), dried ($MgSO_4$) and evaporated *in vacuo* to dryness. The residue is coevaporated with toluene (2x100 ml). The residue is chromatographed over a silica gel column (4.5x25 cm) using toluene/AcOEt (gradient of AcOEt from 0 to 10%) as eluent to give compound 9, 2.10 g (40%), as a colorless oil. **MS**: [M+1]=573&575; [M+1+NH_3]=590&592. **^{1}H-NMR** ($CDCl_3$): 7.45 (q, 1H, ^4J=1.0 Hz, H-6, Thy); 7.40-7.10 (gm, 10H, Phe ,BOM&Bzl); 6.33 (m, 1H, $^3J_{1'-2'}$=6.2&7.2 Hz, H-1', dT); 5.49 (s, 2H, $N\underline{CH_2}$, BOM); 4.69 (s, 2H, $O\underline{CH_2}$, BOM); 4.59&4.49 (d&d, 2H, ^2J=11.8 Hz, OCH_2, Bzl); 4.19 (gm, 2H,, H-3', -4', dT); 3.74 (dd, 1H, $^2J_{5'-5''}$=10.6 Hz, $^3J_{5'-4'}$=2.5 Hz, H-5', dT); 3.61 (m, *, $O\underline{CH_2}CH_2CH_2Br$); 3.57 (m, 3H with *, H-5''); 3.43 (t, 2H, $OCH_2\underline{CH_2}CH_2Br$), 2.50 (m, 1H, $^2J_{2'-2''}$=13.6 Hz, $^3J_{3'-2'}$=2.8 Hz, H-2', dT); 2.11 (m, **, $OCH_2CH_2\underline{CH_2}Br$); 1.97 (m, 3H with **, H-2'', dT); 1.91 (d, 3H, CH_3, Thy). **^{13}C-NMR** ($CDCl_3$): 163.3 (C-4, Thy);150.8 (C-2, Thy); 137.9 (C-1, Phe, BOM); 137.3 (C-1, Phe, Bzl), 134.2 (C-6, Thy); 128.4&128.2&127.9&127.9&127.6& 127,5 (C-2, C-3, C-4, Phe, BOM&Bzl); 109.8 (C-5, Thy); 85.8 (C-1', dT); 83.7 (C-4', dT); 78.6 (C-3', dT); 72.1 (OCH_2, BOM); 71.4 (OCH_2, Bzl); 70.8 (C-5', dT); 70.4 (NCH_2, BOM); 69.9 ($O\underline{CH_2}CH_2CH_2Br$); 37.9 (C-2', dT); 32.4 ($OCH_2\underline{CH_2}CH_2Br$); 29.82

($OCH_2CH_2\underline{C}H_2Br$); 13.3 ($CH_3$, Thy).

Example 10

3-N-Benzyloxymethyl-5'-O-(tetrahydropyran-2-yl)-3'-O-[(4-N-trifluoroacetyl)-1,7-heptaza-4-nediyl]thymidylyl-(3'→5')-3-N-benzyloxymethyl-3'-O-(benzyl)thymidine, 10

To a stirred solution of compound 6 (2.00 g, 3.3 mmole) in anhydrous DMF (10 ml) is added a suspension of 60% NaH (140 mg, 3.5 mmole) and after 1 hour a solution of compound 9 (2.00 g, 3.5 mmole) in anhydrous DMF (5 ml). The resulting mixture is stirred overnight at 50°C, and cooled to ambient temperature. Another portion of 60% NaH (40 mg, 1 mmole) is added and after one hour a solution of compound 9 (600 mg, 1.0 mmole) in anhydrous DMF (2 ml) is added. The resulting mixture is stirred overnight at 50°C, cooled to ambient temperature and the DMF is evaporated *in vacuo* to dryness. The residue is dissolved in AcOEt (100 ml), washed successively with water (1x30 ml) and brine (2x30 ml), dried ($MgSO_4$) and evaporated in vacuo to dryness. The residue is chromatographed over a silica gel column (2.5x25 cm) using toluene/AcOEt (gradient of AcOEt from 10 to 50%) as eluent to give compound **10**, 2.99 g (83%), as a white foam. ^{1}**H-NMR** ($CDCl_3$): 7.61&7.53(m&m, 1H, H-6, dT_1); 7.48&7.36(m&m, *, H-6, dT_3);7.40-7.20 (gm, 16H with *, Phe ,BOM); 6.34 (m, 2H, H-1', dT_1&dT_3); 5.48 (s, 4H, $N\underline{C}H_2$, BOM); 4.69 (s, 2H, $O\underline{C}H_2$, BOM); 4.62 (m, **, H-1, THP); 4.54 (m, 3H with **, OCH_2, Bzl); 4.19 (m, ***, H-4', dT_1&dT_3); 4.19&4.08&4.03 (m&m&m, 4H with***, H-3', dT_1&dT_3); 4.00-3.40 (gm, 14H, H-5', dT_1&dT_3; H-5, THP; $O\underline{C}H_2CH_2\underline{C}H_2N(COCF_3)\underline{C}H_2CH_2\underline{C}H_2O$);; 2.45 (m, 2H, H-2', dT_1&dT_3); 2.00 (m, ****, H-2', dT_1&dT_3); 1.95&1.93&1.91(s&s&s, ****, CH_3, Thy); 1.85 (m, ****, $OCH_2\underline{C}H_2CH_2N(COCF_3)CH_2\underline{C}H_2CH_2O$);

1.80&1.56 (m&m, 18H with ****, H-2,-3,-4, THP). ^{13}C-NMR ($CDCl_3$): 163.36&163.31&163.26 (C-4, dT_1&dT_3); 156.6 (m, $\underline{C}(=O)CF_3$); 150.87&150.85&150.81&150.75 (C-2, dT_1&dT_3); 137.88&137.86&137.84 (C-1, Phe, BOM); 137.4&137.3 (C-1, Phe, Bzl); 134.42&134.30&134.1& 134.06 (C-6, dT_1&dT_3); 128.4-127.5 (gs, C-2, C-3, C-4, Phe, BOM); 116.4 (q, $^1J_{C-F}$=288.1 Hz, $C(=O)\underline{C}F_3$); 110.10&100.04&109.98&109.87 (C-5, dT_1&dT_3); 100.0&99.1&98.9 (C-1, THP); 85.82&85.72&85.70 (C-1', dT_1&dT_3); 83.67&83.62&85.55&83.47 (C-4', dT_1&dT_3); 80.55&80.46 (C-3', dT_1); 79.13&78.83&78.74 (C-3', dT_3); 72.0 (OCH_2, BOM); 70.3 (NCH_2, BOM); 71.34&71.30&71.30 (OCH_2, Bzl); 71.12&70.92&70.86 (C-5', dT_3); 70.3 (NCH_2, BOM); 69.44&68.42&68.36 ($O\underline{C}H_2CH_2CH_2N$, dT_3); 68.0&67.9&67.6 ($O\underline{C}H_2CH_2CH_2N$, dT_1); 66.44&66.39&65.91&65.84 (C-5', dT_1); 63.42&62.86& 62.71 (C-5, THP); 45.2&44.8&44.1 ($\underline{C}H_2N(COCF_3)\underline{C}H_2$); 37.78&37.63&37.58 (C-2', dT_1&dT_3); 32.23&29.03&28.90&27.14 ($OCH_2\underline{C}H_2CH_2N(COCF_3)CH_2\underline{C}H_2CH_2O$); 30.6&30.5 (C-2, THP); 25.11&25.06 (C-4, THP); 20.1&19.7&19.6 (C-3, THP); 13.3&13.2&13.1&13.0 (CH_3, Thy). ^{19}F-NMR ($CDCl_3$): -69.12&69.16 (s&s, $C\underline{F}_3$).

Example 11

5'-O-(tetrahydropyran-2-yl)-3'-O-[(4-N-trifluoroacetyl)-1,7-heptaza-4-nediyl]thymidylyl-(3'→5')-thymidine, 11

To a stirred solution of compound **10** (2.18 g, 2.0 mmole) in a mixture of AcOEt/MeOH (1/1) (50 ml) is added 10% Pd/C (Degussa type, Aldrich) (1.1 g). The flask is evacuated and then flushed 3 times with hydrogen and finally filled with hydrogen at 40 to 50 psi. The resultant suspension was stirred vigrously at 23°C for 14 hours. The suspended material is filtered off through a

pad of Celite in a Buchner funnel and then the funnel is rinsed several times with a small portion of AcOEt and MeOH. The combined filtrate and washings is evaporated *in vacuo* to dryness. The residue is chromatographed over a silica gel column (2.5x25 cm) using 3% MeOH/$CHCl_3$ as eluent to give compound **11**, 1.32 g (87%), as a white foam. **MS**: [M+1]=762; [M+1+NH_3]=779. **^{1}H-NMR** ($CDCl_3$): 9.96&9.87&9.75&9.67 (bs&bs&bs&bs, 2H, N<u>H</u>, Thy); 7.64&7.56&7.54&7.45 (bs&bs&bs&bs, 2H, H-6, dT_1&dT_3); 6.32 (gm, 2H, H-1', dT_1&dT_3); 4.65&4.57 (bs&bs, *, H-1, THP); 4.51 (m, 2H with *, H-3', dT_3); 4.19 (m, 1H, H-4', dT_1); 4.09 (m, **, H-4', dT_3); 4.07 (m, **, H-3', dT_1); 4.1-3.40 (gm, 16H with **, H-5', dT_1&dT_3; H-5, THP; O<u>C</u>H_2CH_2<u>C</u>H_2N(COCF_3)<u>C</u>H_2CH_2<u>C</u>H_2O); 2.41 (m, 2H, H-2', dT); 2.15&2.00 (m&m, ***, H-2', dT); 1.94&1.90 (s&s, ***, CH_3, dT_1&dT_3); 1.90 (m, ***, OCH_2<u>C</u>H_2CH_2N(COCF_3)CH_2<u>C</u>H_2CH_2O); 1.80&1.59 (m&m, 18H with ***, H-2,-3,-4, THP). **^{13}C-NMR** ($CDCl_3$): 164.1&164.0 (C-4, dT_1&dT_3); 156.8 (m, <u>C</u>(=O)CF_3); 150.9&150.6 (C-2, dT_1&dT_3); 135.9&135.7&135.6& 135.3 (C-6, dT_1&dT_3); 116.4 (q, $^1J_{C-F}$=290.3 Hz, C(=O)<u>C</u>F_3); 111.3&111.2&109.98&110.8&110.7 (C-5, dT_1&dT_3); 100.1&99.1&99.0 (C-1, THP); 85.7&85.6 (C-4', dT_3); 85.22&85.12&85.08 (C-1', dT_1&dT_3); 83.65&83.49 (C-4', dT_1); 80.94&80.77 (C-3', dT_1); 71.9&71.6 (C-3', dT_3); 71.0&70.5&70.86&68.4&68.2&67.8&66.5&66.0&65.5 (C-5', dT_3; O<u>C</u>H_2CH_2CH_2N, dT_1&dT_3); 66.44&66.39&65.91&65.84 (C-5', dT_1); 63.5&63.0& 62.8 (C-5, THP); 45.6&45.2&44.5 (<u>C</u>H_2N(COCF_3)<u>C</u>H_2); 40.5 (C-2', dT_3); 37.9&37.7 (C-2', dT_1); 30.72&30.66 (C-2, THP); 29.14&29.05&27.09& 26.98 (OCH_2<u>C</u>H_2CH_2N(COCF_3)CH_2<u>C</u>H_2CH_2O); 25.2&25.1 (C-4, THP); 20.2&19.8&19.7 (C-3, THP); 12.6&12.5&12.3 (CH_3, Thy). **^{19}F-**

NMR ($CDCl_3$): -69.25 (s, $C\underline{F}_3$).

Example 12

5'-O-(tetrahydropyran-2-yl)-3'-O-(1,7-heptaza-4-nediyl)thymidylyl-(3'→5')-thymidine, 12

A solution of compound **11** (1.14 g, 1.5 mmole) in 20 ml of a 3:1 mixture of 12% NH_3/EtOH - 28% NH_4OH is kept at 65°C overnight. The reaction mixture is cooled and evaporated *in vacuo* to dryness and the residue is chromatographed over a silica gel column (4.5x25 cm) using $CHCl_3$/MeOH/28%NH_4OH (95/5/0.5) as eluent to give compound **12**, 0.96 g (96%), as a white solid. MS: [M+1]=666. ^{1}H-NMR ($CDCl_3$): 7.64&7.55 (bs&bs, 2H, H-6, dT_1&dT_3); 6.30 (gm, 2H, H-1', dT_1&dT_3); 4.66&4.59 (bs&bs, 1H, H-1, THP); 4.49 (m, 1H, H-3', dT_3); 4.20 (m, 1H, H-4', dT_1); 4.06 (m, *, H-4', dT_3); 4.00 (m, *, H-3', dT_1); 3.94&3.55 (m&m, *, H-5', dT_1); 3.80&3.52 (m&m,*,; H-5, THP); 3.58 (m, *, H-5', dT_3); 3.52 (m, 12H with *, $O\underline{CH}_2CH_2CH_2NHCH_2CH_2\underline{CH}_2O$) ; 2.75 (gm, 4H, $OCH_2CH_2\underline{CH}_2NH\underline{CH}_2CH_2CH_2O$); 2.38 (gm, 2H, H-2', dT_1&dT_3); 2.20 (m, 1H, H-2', dT_3); 2.01 (m, **, H-2', dT_1);1.93&1.90 (s&s, **, CH_3, dT_1&dT_3); 1.75 (m, **, $OCH_2\underline{CH}_2CH_2NHCH_2\underline{CH}_2CH_2O$); 1.70&1.57 (m&m, 17H with **, H-2,-3,-4, THP). ^{13}C-NMR ($CDCl_3$): 164.4&164.3 (C-4, dT_1&dT_3); 150.83&150.75 (C-2, dT_1&dT_3); 136.0 (C-6, dT_3); 135.7&135.5(C-6, dT_1); 111.02&110.95 (C-5, dT_1); 110.7 (C-5, dT_3); 100.0&99.0 (C-1, THP); 85.6 (C-4', dT_3); 85.2&85.1 (C-1', dT_1&dT_3); 83.8&83.7 (C-4', dT_1); 80.5&80.3 (C-3', dT_1); 71.7 (C-3', dT_3); 70.6&69.9 (C-5', $O\underline{CH}_2CH_2CH_2N$, dT_3); 68.16&67.8 ($O\underline{CH}_2CH_2CH_2N$, dT_1); 67.72&67.67 (C-5', dT_1); 63.3&62.8 (C-5, THP); 47.1&47.0 ($\underline{CH}_2NH\underline{CH}_2$); 40.6 (C-2', dT_3); 37.84&37.78 (C-2', dT_1); 30.7 (C-2, THP); 29.45

($NCH_2\underline{C}H_2CH_2O$, dT_3); 29.2&27.09&26.98 ($OCH_2\underline{C}H_2CH_2N$, dT_1); 25.2 (C-4, THP); 20.1&19.7 (C-3, THP); 12.7 (CH_3, dT_3); &12.6&12.4 (CH_3, dT_1).

Example 13

5'-O-(tetrahydropyran-2-yl)-3'-O-(4-N-[(thymine-1-yl)acetyl]-1,7-heptaza-4-nediyl)thymidylyl-(3'→5')-thymidine, 13a

A solution of compound **12** (333 mg, 0.50 mmole), pentafluorophenyl ester of thymine-1-ylacetic acid (210 mg, 0.60 mmole) and DIPEA (0.6 mmole) in anhydrous DMF (3.0 ml) is kept at ambient temperature for 2 hours and the DMF is evaporated. The residue is chromatographed over a silica gel column (2.5x25 cm) using $CHCl_3$/MeOH/28%NH_4OH (97/3/0.1) as eluent to give compound **13a**, 407 mg (98%), as a white solid. MS: [M+1]=832, [M+NH_3]=848, [M+1+NH_3]=849. ^{1}H-NMR (CD_3OD): 7.76&7.68&7.65&7.58 (m&m&m&m, 2H, H-6, dT_1&dT_3); 7.31&7.28 (1H, H-6, Thy_2); 6.25 (gm, 2H, H-1', dT_1&dT_3); 4.67&4.62 (s&s, *, C(=O)$\underline{C}H_2$Thy); 4.64 (m, 3H with *, H-1, THP); 4.39 (m, 1H, H-3', dT_3); 4.20 (m, **, H-4', dT_1); 4.15 (m, 2H with **, H-3', dT_1); 4.00 (m, ***, H-4', dT_3); 3.95&3.64 (m&m, ***, H-5', dT_1); 3.89&3.52 (m&m,***, H-5, THP); 3.60 (m, ***, H-5', dT_3); 3.70-3.40 (gm, 15H with ***, $O\underline{C}H_2CH_2\underline{C}H_2N$); 2.40 (m, 1H, H-2', dT_1); 2.24 (m, ****, H-2', dT_3); 2.14 (m, 3H with ****, H-2', dT_1); 1.90 (gm, *****, $OCH_2\underline{C}H_2CH_2N$); 1.89&1.88&1.86 (s&s&s, *****, CH_3, Thy); 1.76&1.56 (m&m, 19H with *****, H-2,-3,-4, THP). ^{13}C-NMR (CD_3OD): 175.5&175.3 ($\underline{C}$(=O)CH_2Thy); 168.7&168.7, 167.0&166.9, 166.2&166.1 (C-4, Thy); 155.0&154.6, 153.02&152.98, 152.25&152.19 (C-2, Thy); 144.1 (C-6, Thy_2); 137.9&137.8&137.5 (C-6, dT_1&dT_3); 111.6&111.4&110.7 (C-5,

Thy); 101.4&100.3 (C-1, THP); 87.5&87.1 (C-4', dT_3); 86.6&86.4, 85.6&85.4 (C-1', dT_1&dT_3); 85.2&83.7 (C-4', dT_1); 81.8&81.7 (C-3', dT_1); 72.9&72.5 (C-3', dT_3); 71.9&69.9&68.9&67.7&66.5 (C-5', dT_1&dT_3, O$\underline{C}H_2CH_2CH_2N$,); 64.4&63.6&63.5 (C-5, THP); 50.0 (C(=O)$\underline{C}H_2$Thy); 45.3&44.8&44.7 (-$\underline{C}H_2$N (C(=O)CH_2Thy)$\underline{C}H_2$-); 41.2&41.0&38.4&36.4&36.3&34.9&34.3(C-2', dT_1&dT_3); 31.8 (C-2, THP); 29.8&29.6&28.8 ($NCH_2\underline{C}H_2CH_2O$, dT_1&dT_3); 26.5 (C-4, THP); 21.3&20.7 (C-3, THP); 12.9&12.7&12.6&12.4 (CH_3, Thy).

Example 14

5'-O-(tetrahydropyran-2-yl)-3'-O-{4-N-[(4-N-(isobutyryl)cytidine-1-yl)acetyl]-1,7-heptaza-4-nediyl}thymidylyl-(3'→5')-thymidine, 13b

A solution of compound **12** (333 mg, 0.50 mmole), pentafluorophenyl ester of [4-N-(isobutyryl)cytidine-1-yl]acetic acid (243 mg, 0.60 mmole) and DIPEA (0.6 mmole) in anhydrous DMF (3.0 ml) is kept at ambient temperature for 2 hours and the DMF is evaporated. The residue is chromatographed over a silica gel column (2.5x20 cm) using $CHCl_3$/MeOH (gradient of MeOH from 0,to 7%) as eluent to give compound **13b**, 422 mg (95%), as a white solid. The structure is confirmed by ^{1}H- and ^{13}C-NMR.

Example 15

5'-O-(tetrahydropyran-1-yl)-3'-O-{4-N-[(6-N-(phenoxyacetyl)adenine-9-yl)acetyl]-1,7-heptaza-4-nediyl}thymidylyl-(3'→5')-thymidine, 13c

A solution of compound **12** (333 mg, 0.50 mmole), pentafluorophenyl ester of [6-N-(phenoxyacetyl)adenine-7-yl]acetic acid (296 mg, 0.60 mmole) and DIPEA (0.6 mmole) in anhydrous DMF (3.0 ml) is kept at ambient temperature for 2 hours and the DMF is evaporated. The residue is

chromatographed over a silica gel column (2.5x20 cm) using $CHCl_3$/MeOH (gradient from 0 to 7%) as eluent to give compound **13c**, 412 mg (87%), as a white solid. The structure is confirmed by ^1H- and ^{13}C-NMR.

Example 16

3'-O-{[4-N-(thymine-1-yl)acetyl]-1,7-heptaza-4-nediyl}thymidylyl-(3'→5')-thymidine, 14a

A solution of compound **13a** (300 mg, 0.40 mmole) and PPTS (300 mg, 0.4 mmole) in anhydrous EtOH (6 ml) is kept at 35°C overnight. The reaction mixture is chromatographed over a Sephadex LH-20 column (3.0x120 cm) using MeOH as eluent with a flow rate of 10 ml/h to give compound **14a**, 287 mg (96%), as a white solid. **MS**: [M+1]=748, [M+1+NH_3]=763. **^1H-NMR** (CD_3OD): 7.68&7.59&7.50 (m&m&m, 2H, H-6, dT_1&dT_3); 7.23&7.20 (1H, H-6, Thy_2); 6.14 (gm, 2H, H-1', dT_1&dT_3); 4.58&4.55 (s&s, 2H, C(=O)$\underline{CH_2}$Thy); 4.30 (m, 1H, H-3', dT_3); 4.09&3.98 (m, *, H-3', dT_1); 3.95-3.85 (gm, 3H with *, H-4', dT_1&dT_3); 3.70-3.50 (gm, **, H-5', dT_1&dT_3); 3.60-3.30 (gm, 12H with **, O$\underline{CH_2}CH_2\underline{CH_2}$N); 2.35-1.95 (gm, 4H, H-2', dT_1&dT_3); 1.95-1.65 (gm, ***, $OCH_2\underline{CH_2}CH_2N$); 1.78&1.77&1.76 (s&s&s, ***, CH_3, Thy). **^{13}C-NMR** (CD_3OD): 168.9&168.8, 167.1&167.0, 166.4&166.3 (C-4, Thy); 153.14&153.03, 152.39&152.34&152.27 (C-2, Thy); 144.2 (C-6, Thy_2); 139.1&138.0 (C-6, dT_1&dT_3); 111.70&111.67&111.61&111.46&110.76 (C-5, Thy); 87.6&87.1&86.6 (C-4', dT_1&dT_3); 86.5&86.4&86.3 (C-1', dT_1&dT_3); 85.2&83.7 (C-4', dT_1); 81.2&81.0 (C-3', dT_1); 72.9&72.4 (C-3', dT_3); 71.9&69.9&68.8&67.8&66.7 (C-5', dT_3, O$\underline{CH_2}CH_2CH_2N$,); 63.3 (C-5', dT_1); 50.1&49.9 (-C(=O)$\underline{CH_2}$Thy); 45.4&45.2&44.8 (-$\underline{CH_2}$N(C(=O)CH_2Thy)$\underline{CH_2}$-); 41.2&41.0&38.1 (C-2', dT_1&dT_3); 29.8&29.5&28.8 ($NCH_2\underline{CH_2}CH_2O$, dT_1&dT_3);

12.9&12.6&12.4 (CH_3, Thy).

Example 17

3'-O-{[4-N-(cytidine-1-yl)acetyl]-1,7-heptaza-4-nediyl}thymidylyl-(3'→5')-thymidine, 14b

A solution of compound **13b** (355 mg, 0.40 mmole) in 5 ml of EtOH saturated with NH_3 is kept at ambient temperature overnight. The reaction mixture is evaporated *in vacuo* to dryness. The residue and PPTS (300 mg, 0.4 mmole) in anhydrous EtOH (6 ml) is kept at 35°C overnight. The reaction mixture is chromatographed over a Sephadex LH-20 column (3.0x120 cm) using MeOH as eluent with a flow rate of 10 ml/h to give compound **14b**, 280 mg (87%).

Example 18

3'-O-{4-N-[(adenine-9-yl)acetyl]-1,7-heptaza-4-nediyl}thymidylyl-(3'→5')-thymidine, 14c

Compound **13c** (380 mg, 0.40 mmole) is treated as described in Example 17 to give compound **14c**, 240 mg (82%). The structure is confirmed by ^{1}H-NMR.

Example 19

5'-O-(p-anisyldiphenylmethyl)-3'-O-{[4-N-(thymine-1-yl)acetyl]-1,7-heptaza-4-nediyl}thymidylyl-(3'→5')-thymidine, 15

A solution of compound **13a** (225 mg, 0.30 mmole) and PPTS (300 mg, 0.4 mmole) in anhydrous EtOH (6 ml) is kept at 35°C overnight. Anhydrous Py (20 ml) was added to the reaction mixture. The reaction mixture is evaporated *in vacuo* and then coevaporated with anhydrous Py (3x10 ml), dissolved in anhydrous Py (5 ml) and MMT-Cl (110 mg, 0.36 mmole) is added. The reaction mixture is kept overnight at ambient temperature, then MeOH (100 µl) is added and after 30 minutes the reaction mixture is poured in $CHCl_3$ (50 ml).

The organic layer is washed with $NaHCO_3$ (3x15 ml), brine (2x15 ml), dried ($MgSO_4$), evaporated *in vacuo* to dryness, and the residue is chromatographed over a silica gel column (2.5x10 cm) using $CHCl_3$/MeOH/28%NH_4OH (97/2/0.1) as eluent to give compound **15**, 279 mg (91%), as a white foam. **^{1}H-NMR** (acetone-d_6): 7.66&7.60&7.59 (m&m&m, 2H, H-6, dT_1&dT_3); 7.50-7.20 (gm, *, Phe, MMT); 7.24 (m, 13H with*, H-6, Thy_2); 6.92 (m, 2H, Phe, MMT); 6.29 (gm, 2H, H-1', dT_1&dT_3); 4.65 (m, 2H, C(=O)$\underline{CH_2}$Thy); 4.48 (m, 1H, H-3', dT_3); 4.34&4.27 (m, 1H, H-3', dT_1); 4.17&4.11 (m&m, 1H, H-4',dT_1); 4.00 (m&m, 1H, H-4',dT_3); 3.78 (s, 3H, OCH_3, MMT); 3.75&3.62 (m&m, **, H-5', dT_3); 3.75-3.30 (gm, 12H with **, O$\underline{CH_2}CH_2\underline{CH_2}$N); 3.38 (m, **, H-5', dT_3); 2.38 (m, ***, H-2', dT_1); 2.22 (m, 4H with ***, H-2', dT_3); 2.05-1.75 (gm, ****, $OCH_2\underline{CH_2}CH_2$N); 1.83&1.81&1.79&1.77&1.48 (s&s&s, 13H with ****, CH_3, Thy). **^{1}H-NMR** (DMF-d_7): 7.68&7.66 (m&m, 2H, H-6, dT_1&dT_3); 7.60-7.20 (gm, 13H, Phe, MMT; H-6, Thy_2); 6.98 (m, 2H, Phe, MMT); 6.34 (gm, 2H, H-1', dT_1&dT_3); 5.44 (t, 1H, 3'-OH, dT_3); 4.72 (bs, 2H, C(=O)$\underline{CH_2}$Thy); 4.43 (m, 1H, H-3', dT_3); 4.36&4.30 (m, 1H, H-3', dT_1); 4.18&4.11 (m&m, 1H, H-4',dT_1); 4.01 (m&m, 1H, H-4',dT_3); 3.82 (s, *, OCH_3, MMT); 3.78&3.62 (m&m, *, H-5', dT_3); 3.40 (m, *, H-5', dT_1); 3.75-3.30 (gm, 15H with *, O$\underline{CH_2}CH_2\underline{CH_2}$N); 2.44 (m, 2H, H-2', dT_1); 2.25 (m, 2H, H-2', dT_3); 2.00-1.70 (gm, **, $OCH_2\underline{CH_2}CH_2$N); 1.86&1.84&1.81&1.79&1.56 (s&s&s&s&s, 13H with **, CH_3, Thy). **^{13}C-NMR** (DMF-d_7), only proton correlated carbons: 142.8 (C-6, Thy_2); 136.2&136.0 (C-6, dT_1&dT_3); 130.6&128.6&128.2&127.3&113.5 (Phe, MMT); 86.3&86.1 (C-4', dT_3); 84.7&84.5 (C-1', dT_1&dT_3); 83.7 (C-4', dT_1); 80.0&79.8 (C-3', dT_1); 71.8&71.6 (C-3', dT_3); 71.1 (C-5', dT_3);

&68.9&67.9&67.0&65.9 ($O\underline{C}H_2CH_2CH_2N$,); 64.6&64.5 (C-5', dT_1); 55.2 (OCH_3, MMT); 48.6 ($-C(=O)\underline{C}H_2Thy$); 43.9&43.4&43.2 ($-\underline{C}H_2N(C(=O)CH_2Thy)\underline{C}H_2-$); 39.9 (C-2', dT_3); 36.9 (C-2', dT_1); 29.0&28.8&28.0 ($NCH_2\underline{C}H_2CH_2O$, dT_1&dT_3); 12.2&11.9&11.8 (CH_3, Thy).

Example 20

5'-O-(p-anisyldiphenylmethyl)-3'-O-{[4-N-(thymine-1-yl)acetyl]-1,7-heptaza-4-nediyl}thymidylyl-(3'→5')-3'-O-(succinoyl)thymidine, 16

Compound **15** (252 mg, 0.25 mmole) is coevaporated with anhydrous Py (3x5 ml) and dissolved in Py (3 ml). Succinic anhydride (100 mg, 1 mmole) and DMAP (30 mg, 0.25) is added, and reaction mixture is kept at ambient temperature overnight. A 1:3 mixture of H_20/Py (4 ml) is added and kept 1 hour at ambient temperature. The reaction mixture is evaporated, the residue is dissolved in $CHCl_3$ (25 ml), washed successively with cold 0.5 M citric acid (3x10 ml) and brine (2x10 ml), dried ($MgSO_4$) and evaporated *in vacuo* to dryness. The residue is coevaporated with anhydrous dioxane (2x5 ml) and lyophylized from anhydrous dioxane (3 ml) to give compound **16**, 270 mg (97%). **^{1}H-NMR** (acetone-d_6): 7.66&7.64&7.60 (m&m&m, 2H, H-6, dT_1&dT_3); 7.49 (m, 4H, Phe, MMT); 7.40-7.20 (gm, *, Phe, MMT); 7.23 (m, 9H with *, H-6, Thy_2); 6.92 (m, 2H, Phe, MMT); 6.29 (gm, 2H, H-1', dT_1&dT_3); 5.35 (m, 1H, H-3', dT_3);4.65 (m, 2H, $C(=O)\underline{C}H_2Thy$); 4.32 (m, 1H, H-3', dT_1); 4.16 (gm, 2H, H-4',dT_1); 4.00 (m&m, 1H, H-4', dT_1&dT_3); 3.79 (s, **, OCH_3, MMT); 3.90-3.20 (gm, 15H with **, H-5', dT_1&dT_3; $O\underline{C}H_2CH_2\underline{C}H_2N$); 2.63&2.62 (s&s, 4H, $-OOCCH_2CH_2COOH$); 2.35 (gm, 4H, H-2', dT_1&dT_3); 1.95-1.75 (gm, ***, $OCH_2\underline{C}H_2CH_2N$); 1.84&1.82&1.79&1.77&1.48 (s&s&s, 13H with ***, CH_3, Thy).

^{13}C-NMR (acetone-d_6), only proton correlated carbons: 142.7 (C-6, Thy_2); 136.0 (C-6, dT_1&dT_3); 129.9&128.9&128.4&127.5&113.7 (Phe, MMT); 85.2&85.1&84.9&84.1&83.9 (C-1', C-4', dT_1&dT_3); 80.4&80.2 (C-3', dT_1); 76.2&75.6 (C-3', dT_3); 71.2 (C-5', dT_3); 69.2&67.9&67.2&66.0 ($OCH_2CH_2CH_2N$,); 64.7&64.6 (C-5', dT_1); 55.2 (OCH_3, MMT); 48.8 (-C(=O)CH_2Thy); 44.3&44.2&43.7&43.5 (-CH_2N(C(=O)CH_2Thy)CH_2-); 37.5 (C-2', dT_1&dT_3); 29.30&29.28&29.20&28.74&28.26 (-$NCH_2CH_2CH_2O$- & -$OOCCH_2CH_2COOH$); 12.4&12.30&11.90&11.80 (CH_3, Thy).

Example 21

CPG bound trinucleoside 16, 17

Trinucleoside 16 is converted to the succinimide ester and treated with long chain alkylamine CPG (500 Å, Sigma). The resulting CPG 17 with a loading of 15-20 µmole/g is used for solid phase oligonucleotide synthesis according standard protocols.

Example 22

5'-O-(p-anisyldiphenylmethyl)-3'-O-{[4-N-(thymine-1-yl)acetyl]-1, 7-heptaza-4-nediyl}thymidylyl-(3'→5')-3'-[(ß-cyanoethoxy)-N-(diisopropyl)phosphiryl]thymidine, 18

Compound 15 (504 mg, 0.5 mmol) is dissolved in anhydrous dichloromethane (7 ml) under an argon atmosphere. Diisopropylethylamine (0.22 ml, 1.23 mmol) is added and the reaction mixture cooled to -30°C. Chloro(diisopropylamino)-ß-cyanoethoxyphosphine (0.75 mmol) is added to the reaction mixture and the reaction mixture is allowed to warm to 20°C and stirred for 4 hours. Ethylacetate (40 ml) and triethylamine (0.5 ml) are added and the solution is washed with brine solution (3 x 20 ml). The organic phase is separated and dried over sodium

sulfate. After filtration of the solids the solvent is evaporated *in vacuo* at 20°C to an oil that is then purified by column chromatography using silica and a solvent such as toluene-ethyl acetate-triethylamine (20:80:1) as eluent. The fractions are then evaporated *in vacuo* (1 torr) at 20°C and the residue is evaporated with anhydrous pyridine and the rest is lyophilized from benzene-acetonitrile (1:1) to yield title compound **18**.

Example 23

3-N-Benzyloxymethyl-3'-O-[3-N-(trifluoroacetyl)-3-N-(*tert*-butoxycarbonylmethyl)-3-aminopropyl]-5'-O-(tetrahydropyran-2-yl)thymidine, 19

To a stirred solution of compound **6** (2.00 g, 3.3 mmole) in anhydrous DMF (10 ml) is added a suspension of 60% NaH (140 mg, 3.5 mmole) and after 1 hour *tert*-butyl ester of bromoacetic acid (FW 195.06, d 1.321) (0.68 g, 0.52 ml, 3.5 mmole). The resulting mixture is stirred overnight at 50°C and cooled to ambient temperature. Another portion of 60% NaH (40 mg, 1 mmole) is added and after 1 hour *tert*-butyl ester of bromoacetic acid (0.20 mg, 0.15 ml, 1 mmole) is added. The resulting mixture is stirred overnight at 50°C, cooled to ambient temperature and DMF is evaporated *in vacuo* to dryness. The residue is dissolved in AcOEt (100 ml), washed successively with water (1x30 ml) and brine (2x30 ml), dried ($MgSO_4$) and evaporated *in vacuo* to dryness. The residue is chromatographed over silica gel column (2.5x25 cm) using toluene/AcOEt (gradient of AcOEt from 10 to 50%) as eluent to give compound **19**, 2.10 g (89%), as a white foam. ^{1}H-NMR ($CDCl_3$):

7.60&7.53(m&, 1H, H-6, dT); 7.40-7.20 (gm, 5H, Phe , BOM); 6.30 (m, 1H, H-1', dT); 5.49 (s, 2H, $N\underline{CH_2}$, BOM); 4.85 (m, 2H, $N\underline{CH_2}COOBu^t$); 4.70 (s, 2H, $O\underline{CH_2}$, BOM); 4.64&4.59 (m&m, 1H, H-1, THP); 4.18 (m, *, H-4', dT); 4.15&4.08 (m&m, *, H-3', dT); 4.10-3.40 (gm, 10H with *, H-5', dT; H-5, THP;

$O\underline{CH_2}CH_2\underline{CH_2}N(COCF_3)$-); 2.44 (m, 1H, H-2', dT); 2.02 (m, **, H-2', dT); 1.95&1.91(d&d, **, CH_3, Thy); 1.87 (m, **, $OCH_2\underline{CH_2}CH_2N(COCF_3)$-); 1.80&1.56&1.45 (m&m&m, 21H with ****, H-2,-3,-4, THP; $NCH_2COO\underline{Bu^t}$). ^{19}F-NMR ($CDCl_3$): -69.30 ($C\underline{F_3}$).

Example 24

3'-O-[3-N-(trifluoroacetyl)-3-N-(tert-butoxycarbonylmethyl)-3-aminopropyl]-5'-O-(tetrahydropyran-2-yl)thymidine, 20

To a stirred solution of compound **19** (2.14 g, 3.0 mmole) in AcOEt (50 ml) is added 10% Pd/C (Degussa type, Aldrich) (1.1 g). The flask is evacuated and then flushed 3 times with hydrogen and finally filled with hydrogen at 40 to 50 psi. The resultant suspension was stirred vigorously at 23°C for 14 hours. The suspended material is filtered off through a pad of Celite in a Buchner funnel and then the funnel is rinsed several times with a small portion of AcOEt and MeOH. The combined filtrate and washing is evaporated *in vacuo* to dryness. The residue is chromatographed over a silica gel column (2.5x25 cm) using toluene/AcOEt (gradient of AcOEt from 10 to 70%) as eluent to give compound **20**, 1.59 g (87%), as a white foam. ^{1}H-NMR ($CDCl_3$): 9.96&9.87 (bs&bs, 1H, N$\underline{H}$, Thy);7.60&7.53(m&, 1H, H-6, dT); 6.30 (m, 1H, H-1', dT); 4.80 (m, 2H, $N\underline{CH_2}COOBu^t$); 4.64&4.59 (m&m, 1H, H-1, THP); 4.18 (m, *, H-4', dT); 4.15&4.08 (m&m, *, H-3', dT); 4.10-3.40 (gm, 10H with *, H-5', dT; H-5, THP; $O\underline{CH_2}CH_2\underline{CH_2}N(COCF_3)$-); 2.44 (m, 1H, H-2', dT); 2.02 (m, **, H-2', dT); 1.95&1.91(d&d, **, CH_3, Thy); 1.87 (m, **, $OCH_2\underline{CH_2}CH_2N(COCF_3)$-); 1.80&1.56&1.45 (m&m&m, 21H with **, H-2, -3,-4, THP; $NCH_2COO\underline{Bu^t}$). ^{19}F-NMR ($CDCl_3$): -69.30 ($C\underline{F_3}$).

Example 25

3'-O-[3-N-(*tert*-butoxycarbonylmethyl)-3-aminopropyl]-5'-O-(tetrahydropyran-2-yl)thymidine, 21

A solution of compound **20** (1.82 g, 3.0 mmole) in 20 ml of 3:1 mixture of 12% NH_3/EtOH - 28% NH_4OH is kept at 65°C overnight. The reaction mixture is cooled and evaporated *in vacuo* to dryness and the residue is chromatographed over a silica gel column (4.5x25 cm) using $CHCl_3$/28%NH_4OH (99.5/0.5) (gradient of MeOH from 0 to 7%) as eluent to give compound **21**, 1.47 g (96%), as an oil. **^{1}H-NMR** ($CDCl_3$): 9.95 (bs, 1H, N<u>H</u>, Thy); 7.60 (m, 1H, H-6, dT); 6.30 (m, 1H, H-1', dT); 4.64&4.59 (m&m, 1H, H-1, THP); 4.30-4.00 (gm, 4H, H-4', dT;H-3', dT; -NH<u>CH$_2$</u>COOBu^t); 4.00-3.40 (gm, 6H, H-5', dT; H-5, THP; O<u>CH$_2$</u>CH$_2$CH$_2$NH-); 2.75 (m, 2H, OCH$_2$CH$_2$<u>CH$_2$</u>NH); 2.40 (m, 1H, H-2', dT); 2.02 (m, *, H-2', dT); 1.93 (d, *, CH_3, Thy); 1.75 (m, *, OCH$_2$<u>CH$_2$</u>CH$_2$NH-); 1.70&1.56&1.45 (m&m&m, 21H with *, H-2, -3,-4, THP; -NHCH$_2$COO<u>Bu^t</u>).

Example 26

3'-O-{3-N-[(thymine-1-yl)acetyl]-3-N-(*tert*-butoxycarbonylmethyl)-3-aminopropyl}-5'-O-(tetrahydropyran-2-yl)thymidine, 22a

A solution of compound **21** (1.02 g, 2.00 mmole), pentafluorophenyl ester of thymine-1-ylacetic acid (0.77 g, 2.10 mmole) and DIPEA (2.10 mmole) in anhydrous DMF (10.0 ml) is kept at ambient temperature for 4 hours and the DMF is evaporated. The residue is chromatographed over a silica gel column (2.5x25 cm) using $CHCl_3$/MeOH/28%NH_4OH (97/3/0.1) as eluent to give compound **22**, 1.33 g (98%), as a white solid. **^{1}H-NMR** (CD_3OD): 7.65&7.58 (m&m, 1H, H-6, dT_1); 7.30&7.27 (m&m,1H, H-6, Thy_2); 6.23 (m, 1H, H-1',

dT_1); 4.85-4.60 (gm, 5H, -N$\underline{CH_2}$COOBu^t; -C(=O)$\underline{CH_2}$Thy; H-1, THP); 4.20 (m, *, H-4', dT_1); 4.15 (m, 2H with *, H-3', dT_1); 4.00-3.40 (gm, 8H, H-5', dT_1; H-5, THP; O$\underline{CH_2}CH_2\underline{CH_2}$N); 2.38 (m, 1H, H-2', dT_1); 2.10 (m, 1H, H-2', dT_1); 2.00-1.40 (gm, 23H, OCH$_2\underline{CH_2}$CH$_2$N; CH_3, dT_1&Thy_2; H-2, -3,-4, THP; -NHCH$_2$COO$\underline{Bu^t}$).

Example 27

3'-O-{3-N-[((4-N-(isobutyryl)cytidine-1-yl)acetyl]-3-N-(tert-butoxycarbonylmethyl)-3-aminopropyl}-5'-O-(tetrahydropyran-2-yl)thymidine, 22b

A solution of compound **21** (1.02 g, 2.00 mmole), pentafluorophenyl ester of [4-N-(isobutyryl)cytidine-1-yl]acetic acid (0.85 g, 2.10 mmole) and DIPEA (2.10 mmole) in anhydrous DMF (10.0 ml) is kept at ambient temperature for 4 hours and the DMF is evaporated. The residue is chromatographed over a silica gel column (2.5x25 cm) using $CHCl_3$/MeOH/(gradient of MeOH from 0 to 7%) as eluent to give compound **22b**, 1.42 g (97%), as a white solid.

Example 28

3'-O-{3-N-[((6-N-(phenoxyacetyl)adenine-9-yl)acetyl]-3-N-(tert-butoxycarbonylmethyl)-3-aminopropyl}-5'-O-(tetrahydropyran-2-yl)thymidine, 22c

A solution of compound **21** (1.02 g, 2.00 mmole), pentafluorophenyl ester of [6-N-(phenoxyacetyl)adenine-9-yl]acetic acid (1.04 g, 2.10 mmole) and DIPEA (2.10 mmole) in anhydrous DMF (10.0 ml) is kept at ambient temperature for 4 hours and the DMF is evaporated. The residue is chromatographed over a silica gel column (2.5x25 cm) using $CHCl_3$/MeOH/(gradient of MeOH from 0 to 7%) as eluent to give compound **22c**, 1.51 g (92%), as a white solid.

Example 29

3'-O-{3-N-[(thymine-1-yl)acetyl]-3-N-(carboxymethyl)-3-aminopropyl}-5'-O-(p-anisyldiphenylmethyl)thymidine, 23a

A solution of compound **22a** (1.69 g, 2.5 mmole) in 50% TFA/DCM (50 ml) is kept at ambient temperature for 30 minutes. The reaction mixture is evaporated *in vacuo* and the residue is coevaporated with toluene (2x50 ml), anhydrous Py (3x50 ml), dissolved in anhydrous Py (25 ml), and MMT-Cl (0.93 g, 3.0 mmole) is added. The reaction mixture is kept at ambient temperature overnight and evaporated. The residue is suspended in $CHCl_3$ (150 ml) and washed successively with water (2x50 ml), cold citric acid (3x30 ml) and brine (2x50 ml), dried ($MgSO_4$) and evaporated *in vacuo*. The residue is reprecipitated from $CHCl_3$/hexane. The precipitate is collected and dried *in vacuo* to give compound **23a**, 1.92 g (95%), as a white solid. ^{1}H-NMR (CD_3OD): 7.65&7.58 (m&m, 1H, H-6, dT_1); 7.45-7.10 (gm, 13H, Phe, MMT; H-6, Thy_2); 6.82 (m, 2H, Phe, MMT); 6.20 (m, 1H, H-1', dT_1); 4.90-4.60 (gm, 4H, $-N\underline{CH_2}COOH$; $-C(=O)\underline{CH_2}Thy$); 4.20 (m, *, H-4', dT_1); 4.15 (m, 2H with *, H-3', dT_1); 4.00-3.40 (gm, 9H, H-5', dT_1; OCH_3, MMT; $O\underline{CH_2}CH_2\underline{CH_2}N$); 2.40 (m, 1H, H-2', dT_1); 2.08 (m, 1H, H-2', dT_1); 2.00-1.40 (gm, 8H, $OCH_2\underline{CH_2}CH_2N$; CH_3, dT_1&Thy_2).

Example 30

3'-O-{3-N-[((4-N-(isobutyryl)cytidine-1-yl)acetyl]-3-N-(carboxymethyl)-3-aminopropyl}-5'-O-(p-anisyldiphenylmethyl)thymidine, 23b

Compound **22b** (1.46 g, 2.00 mmol) is treated as described in Example 29 to give the title compound **23b**, 1.47 g (85%).

Example 31

3'-O-{3-N-[(6-N-(phenoxyacetyl)adenine-1-yl)acetyl]-3-N-(carboxymethyl)-3-aminopropyl}-5'-O-(p-anisyldiphenylmethyl)thymidine, 23c

Compound **22c** (1.64 g, 2.00 mmol) is treated as described in Example 29 to give the title compound **23c**, 1.49 g (78%).

Example 32

3'-O-{3-N-[(thymine-1-yl)acetyl]-3-N-(pentafluorophenoxycarbonylmethyl)-3-aminopropyl}-5'-O-(p-anisyldiphenylmethyl)thymidine, 24a

A solution of compound **23a** (1.00 mmol), pentafluorophenol (1.1 mmol) and N,N'-dicyclohexylcarbodimide (1.1 mmol) in DMF (7 ml) is kept at ambient temperature for 1 hour. N,N'-dicyclohexylurea is filtered off and the resulting solution of compound **24a** is used without purification.

Example 33

3'-O-{3-N-[(4-N-(isobutyryl)cytidine-1-yl)acetyl]-3-N-(pentafluorophenoxycarbonylmethyl)-3-aminopropyl}-5'-O-(p-anisyldiphenylmethyl)thymidine, 24b

Compound **23b** (1.00 mmol) is treated as described in Example 32 to give a solution of the title compound **24b**.

Example 34

3'-O-{3-N-[(6-N-(phenoxyacetyl)adenine-1-yl)acetyl]-3-N-(pentafluorophenoxycarbonylmethyl)-3-aminopropyl}-5'-O-(p-anisyldiphenylmethyl)thymidine, 24c

Compound **23c** (1.00 mmol) is treated as described in Example 32 to give a solution of the title compound **24c**.

Example 35

3-N-Benzyloxymethyl-5'-O-(2-cyanoethyl)-3'-O-(benzyl)thymidine, 25

To a stirred solution of compound 7 (2.0 mmole) in anhydrous THF (150 ml) is added a suspension of 60% NaH (60 mg, 1.5 mmole) in one portion and dropwise neat acrylonitrile (5 ml) over 5 minutes. The resulting mixture is stirred at ambient temperature for an additional 1 hour. The mixture is evaporated *in vacuo* to dryness. The residue is dissolved in AcOEt (500 ml). The organic layer is washed with brine (2x250 ml), dried ($MgSO_4$), evaporated *in vacuo* to dryness, and the residue is chromatographed over a silica gel column (4.5x30 cm) using toluene/AcOEt (gradient of AcOEt from 10 to 15%) as eluent to give compound **25**, (92%), as a colorless oil. **^{1}H-NMR** ($CDCl_3$): 7.45-7.15 (gm, 11H, Phe, BOM&Bzl; H-6, dT); 6.40 (m, 1H, H-1', dT); 5.48 (s, 2H, NCH_2, BOM); 4.68 (s, 2H, OCH_2, BOM); 4.53 (m, 2H, OCH_2, Bzl); 4.24 (m, H-3', dT); 4.16 (m, H-4', dT); 3.80-3.55 (gm, 4H, H-5', dT; $O\underline{CH_2}CH_2CN$); 2.56 (t, 2H, $OCH_2\underline{CH_2}CN$); 2.45 (m, 1H, H-2', dT); 2.09 (m, 1H, H-2', dT);1.94 (d, 3H, CH_3, Thy). **^{13}C-NMR** ($CDCl_3$): 163.2 (C-4, Thy); 150.7 (C-2, Thy); 137.8&137.3 (C-1, Phe, BOM; C-1, Bzl); 134.1 (C-6, Thy); 128.3-127.4 (gs, C-2, C-3, C-4, Phe, BOM&Bzl); 117.4 (-$\underline{C}N$); 110.1 (C-5, Thy); 85.4 (C-1', dT); 83.1 (C-4', dT); 78.4 (C-3', dT); 71.9 (OCH_2, BOM); 71.4 (OCH_2, Bzl); 70.7 (C-5', dT); 70.3 (NCH_2, BOM); 65.6 ($O\underline{CH_2}CH_2CN$); 55.2 (OMe, MMT); 37.4 (C-2', dT); 18.7 ($OCH_2\underline{CH_2}CN$); 13.2 (CH_3, Thy).

Example 36

3-N-Benzyloxymethyl-5'-O-(3-trifluoroacetamidopropyl)-3'-O-(benzyl)thymidine, 26

Compound **25** is treated as described in Example 5 to give 3-N-benzyloxymethyl-5'-O-(3-aminopropyl)-3'-O-(benzyl)thymidine, (62%), as a yellowish oil. **^{1}H-NMR**

($CDCl_3$): 7.52 (m,1H, H-6, Thy); 7.40-7.20 (gm, 10H, Phe ,BOM&Bzl); 6.35 (m, 1H, H-1', dT); 5.47 (s, 2H, $N\underline{CH_2}$, BOM); 4.68 (s, 2H, $O\underline{CH_2}$, BOM); 4.52 (m, 1H, $O\underline{CH_2}$, Bzl); 4.16 (gm, 2H, H-4', H-3', dT); 3.70 (m, 1H, H-5', dT); 3.60-3.40 (gm, 3H, $O\underline{CH_2}CH_2CH_2NH_2$; H-5', dT); 2.74 (t, 2H, $OCH_2CH_2\underline{CH_2}NH_2$); 2.44 (m, 1H, H-2', dT); 2.03 (m, 1H, H-2', dT); 1.92 (d, 3H, CH_3, Thy); 1.71 (m, **, $OCH_2CH_2\underline{CH_2}NH_2$). **^{13}C-NMR** ($CDCl_3$): 163.3 (C-4, Thy); 150.8 (C-2, Thy); 137.9&137.4 (C-1, Phe, BOM&Bzl); 134.3 (C-6, Thy); 128.4-127.4 (C-2, C-3, C-4, Phe, BOM&Bzl); 109.9 (C-5, Thy); 85.8 (C-1', dT); 83.7 (C-4', dT); 78.7 (C-3', dT); 72.0 (OCH_2, BOM); 71.2 (OCH_2, Bzl); 70.8 (C-5', dT); 70.3 (NCH_2, BOM); 69.4 ($O\underline{CH_2}CH_2CH_2NH_2$); 39.1 ($OCH_2CH_2\underline{CH_2}NH_2$); 37.8 (C-2', dT); 32.2 ($OCH_2\underline{CH_2}CH_2NH_2$); 13.2 ($CH_3$, Thy).

3-N-Benzyloxymethyl-5'-O-(3-aminopropyl)-3'-O-(benzyl)thymidine is treated as described in Example 6 to give the title compound **26**, (93%). **^{1}H-NMR** ($CDCl_3$): 7.40-7.20 (gm, 12H, Phe , BOM&Bzl; H-6, Thy; $\underline{NH}COCF_3$); 6.29 (m, 1H, H-1', dT); 5.47 (s, 2H, $N\underline{CH_2}$, BOM); 4.68 (s, 2H, $O\underline{CH_2}$, BOM); 4.52 (m, 2H, $O\underline{CH_2}$, Bzl); 4.17 (m, 2H, H-4', H-3', dT); 3.68 (m, 1H, H-5', dT); 3.60-3.30 (gm, 5H, $O\underline{CH_2}CH_2\underline{CH_2}NHCOCF_3$; H-5', dT); 3.50 (m, 10H with **, $OCH_2CH_2\underline{CH_2}NHCOCF_3$); 2.48 (m, 1H, H-2', dT); 2.06 (m, 1H, H-2', dT); 1.89(s, 3H, CH_3, Thy); 1.83 (m, 2H, $OCH_2\underline{CH_2}CH_2NHCOCF_3$). **^{13}C-NMR** ($CDCl_3$): 163.3 (C-4, Thy); 157.1 (q, $^2J_{C-F}$=36.9 Hz, $\underline{C}(=O)CF_3$); 150.7 (C-2, Thy); 137.7&137.3 (C-1, Phe, BOM&Bzl); 134.4 (C-6, Thy); 128.4-127.0 (C-2, C-3, C-4, Phe, BOM); 115.7 (q, $^1J_{C-F}$=287.9 Hz, $C(=O)\underline{C}F_3$); 109.9 (C-5, Thy); 86.0 (C-1', dT); 83.4 (C-4', dT); 78.6 (C-3', dT); 72.0 (OCH_2, BOM); 71.4 (OCH_2, Bzl); 71.0 (C-5', dT); 70.3 (NCH_2, BOM); 69.2 ($O\underline{CH_2}CH_2CH_2NHCOCF_3$); 37.6&37.5

(C-2', dT); 28.6 ($OCH_2CH_2CH_2NHCOCF_3$); 13.1 (CH_3, Thy). ^{19}F-NMR ($CDCl_3$): -76.06 (s, CF_3).

Example 37

3-N-Benzyloxymethyl-5'-O-[3-N-(trifluoroacetyl)-3-N-(*tert*-butoxycarbonylmethyl)-3-aminopropyl]-3'-O-(benzyl)thymidine, 27

Compound **26** (2.00 mmol) is treated as described in Example 23 to give the title compound **27**, (85%).

Example 38

5'-O-[3-N-(trifluoroacetyl)-3-N-(carboxymethyl)-3-aminopropyl]-thymidine, 28

Compound **27** (2.00 mmol) is treated as described in Example 24 to give 5'-O-[3-N-(trifluoroacetyl)-3-N-(*tert*-butoxycarbonylmethyl)-3-aminopropyl]thymidine, (82%). 5'-O-[3-N-(Trifluoroacetyl)-3-N-(*tert*-butoxycarbonylmethyl)-3-aminopropyl]thymidine is treated then as described in Example 29 to remove the tert-butyl protecting group from the carboxyl moiety and to give the title compound **28**, (95%).

Example 39

5'-O-{3-N-[(thymine-1-yl)acetyl]-3-N-(carboxymethyl)-3-aminopropyl)-thymidine, 29a

Compound **28** is treated as described in Example 25 to give 5'-O-[3-N-(carboxymethyl)-3-aminopropyl]thymidine, (97%). 5'-O-[3-N-(Carboxymethyl)-3-aminopropyl]- thymidine is treated then as described in Example 26 to give the title compound **29a**, as a white solid (92%).

Example 40

5'-O-{3-N-[(4-N-(isobutyryl)cytidine-1-yl)acetyl]-3-N-(carboxymethyl)-3-aminopropyl)thymidine, 29b

5'-O-[3-N-(Carboxymethyl)-3-aminopropyl]- thymidine is treated then as described in Example 27 to give the

title compound **29b**, as a white solid (91%).

Example 41

5'-O-{3-N-[(6-N-(phenoxyacetyl)adenine-1-yl)acetyl]-3-N-(carboxymethyl)-3-aminopropyl}thymidine, 29c

5'-O-[3-N-(Carboxymethyl)-3-aminopropyl]- thymidine is treated then as described in Example 28 to give the title compound **29c**, as a white solid (93%).

Example 42

5'-O-{3-N-[(thymine-1-yl)acetyl]-3-N-(carboxymethyl)-3-aminopropyl}--3'-O-(di-p-anisyldiphenylmethyl)thymidine, 30a;

5'-O-{3-N-[(4-N-(isobutyryl)cytidine-1-yl)acetyl]-3-N-(carboxymethyl)-3-aminopropyl}-3'-O-(di-p-anisyldiphenylmethyl)thymidine, 30b;

5'-O-{3-N-[(6-N-(phenoxyacetyl)adenine-1-yl)acetyl]-3-N-(carboxymethyl)-3-aminopropyl}-3'-O-(di-p-anisyldiphenylmethyl) thymidine, 30c

Compound **29** is treated with DMT-Cl in the same way as described in Example 29 to give the title compounds **30a-c**.

Example 43

5'-O-{3-N-[(thymine-1-yl)acetyl]-3-N-(succinimidoxycarbonylmethyl)-3-aminopropyl}--3'-O-(di-p-anisyldiphenylmethyl)thymidine, 31a;

5'-O-{3-N-[(4-N-(isobutyryl)cytidine-1-yl)acetyl]-3-N-(succinimidoxycarbonylmethyl)-3-aminopropyl}-3'-O-(di-p-anisyldiphenylmethyl)thymidine, 31b;

5'-O-{3-N-[(6-N-(phenoxyacetyl)adenine-1-yl)acetyl]-3-N-(succinimidoxycarbonylmethyl)-3-aminopropyl}-3'-O-(di-p-anisyldiphenylmethyl) thymidine, 31c

A solution of compound **30** (1.00 mmol), N-hydroxysuccinimide (1.1 mmol) and N,N'-

dicyclohexylcarbodimide (1.1 mmol) in DMF (7 ml) is kept at ambient temperature for 1 hour. N,N'-dicyclohexylurea is filtered off and resulting solution of compound **31** is used without purification.

5

Example 43a

3-N-Benzyloxymethyl-6'-O-(p-anisyldiphenylmethyl)-5'- homothymidine, 40

5'-Homothymidine (5.12 g, 20 mmol) (Etzold, G. *et al.*, Chemical Communications, (1968), p. 422) is monomethoxytritylated as per the procedure of Example 19 and then benzyloxymethylated and purified as per the procedure of Example 1 to give entitled compound **40** (87%).

Example 44

3-N-Benzyloxymethyl-6-3'-O-(2-cyanoethyl)'-O-(p-anisyldiphenylmethyl)-5'-homothymidine, 41

Compound **40** is cyanoethylated and purified as per the procedure of Example 2 to give compound **41**.

Example 45

3-N-Benzyloxymethyl-3'-O-(2-cyanoethyl)-6'-O-(tetrahydropyran-2-yl)-5'-homothymidine, 42

Compound **41** is deprotected as per the procedure of Example 3 and then treated as per the procedure of Example 4 to give compound **42**.

Example 46

3-N-Benzyloxymethyl-3'-O-(3-aminopropyl)-6'-O-(tetrahydropyran-2-yl)-5'-homothymidine, 43

Compound **42** is reduced and purified as per the procedure of Example 5 to give compound **43**.

Example 47

3-N-Benzyloxymethyl-3'-O-(3-trifluoroacetamidopropyl)-6'-O-(tetrahydropyran-2-yl)-5'-homothymidine, 44

Compound **43** is trifluoroacetylated and purified as per the procedure of Example 6 to give compound **44**.

Example 48

3-N-Benzyloxymethyl-3'-O-[3-N-(trifluoroacetyl)-3-N-(*tert*-butoxycarbonylmethyl)-3-aminopropyl)-6'-O-(tetrahydropyran-2-yl)-5'-homothymidine, 45

Compound **44** is alkylated with tert-butyl ester of bromoacetic acid and purified as per the procedure of Example 23 to give compound **45**.

Example 49

3-N-Benzyloxymethyl-3'-O-[3-N-(trifluoroacetyl)-3-N-(*tert*-butoxycarbonylmethyl)-3-aminopropyl)-6'-bromo-5'-homothymidine, 46

The solution of compound **45** (5 mmol), CBr_4 (7 mmol) and $P(Ph)_3$ (14 mmol) in anhydrous THF (40 ml) is stirred at ambient temperature for 24 hours. The reaction mixture is evaporated *in vacuo* to dryness, the residue coevaporated with toluene (2 x 100 ml) and chromatographed over a silica gel column (4.5x25 cm) using toluene/AcOEt (7:3c1:1) as eluent to give compound **46**, (95%).

Example 50

3-N-Benzyloxymethyl-3'-O-[3-N-(trifluoroacetyl)-3-N-(*tert*-butoxycarbonylmethyl)-3-aminopropyl]-6'-N-(di-*tert*-butyloxycarbonyl)-6'-amino-5'-homothymidine, 47

To a stirred solution of di-tert-butyliminodicarboxylate (1.09 g, 5.0 mmole) in anhydrous DMF (20 ml) is added a suspension of 60% NaH (200 mg, 5.0 mmole) and after 1 hour compound **46** (4.0 mmole). The

resulting mixture is stirred overnight at 50°C, cooled to ambient temperature and DMF is evaporated *in vacuo* to dryness. The residue is dissolved in AcOEt (150 ml), washed successively with water (1x30 ml) and brine (2x30 ml), dried ($MgSO_4$) and evaporated *in vacuo* to dryness. The residue is chromatographed over silica gel column (2.5x25 cm) using toluene/AcOEt (gradient of AcOEt from 10 to 50%) as eluent to give compound **35**.

Example 51

3'-O-[3-N-(*tert*-butoxycarbonylmethyl)-3-aminopropyl]-6'-N-(*tert*-butyloxycarbonyl)-6'-amino-5'-homothymidine, 48

Compound **47** (2.0 mmole) is hydrogenated and purified as per the procedure of Example 11. The product of reaction is treated with10 ml of 3:1 mixture of 12% NH_3/EtOH - 28% NH_4OH at ambient temperature overnight. The reaction mixture is cooled and evaporated *in vacuo* to dryness and the residue is chromatographed over a silica gel column (2.5x25 cm) using $CHCl_3$/MeOH/28%NH_4OH (95/1/0.5) as eluent to give compound **48**.

Example 52

3'-O-{3-N-[(thymine-1-yl)acetyl]-3-N-(*tert*-butoxycarbonylmethyl)-3-aminopropyl}-6'-N-(*tert*-butyloxycarbonyl)-6'-amino-5'-homothymidine, 49a

Compound **48** is treated as described in Example 26 to give the title compound **49a**, as a white solid (95%).

Example 53

3'-O-{3-N-[(4-N-(isobutyryl)cytidine-1-yl)acetyl]-3-N-(*tert*-butoxycarbonylmethyl)-3-aminopropyl}-6'-N-(*tert*-butyloxycarbonyl)-6'-amino-5'-homothymidine, 49b

Compound **48** is treated as described in Example 27 to give the title compound **49b**, as a white solid (95%).

Example 54

3'-O-{3-N-[(thymine-1-yl)acetyl]-3-N-(carboxymethyl)-3-aminopropyl}-6'-N-(p-anisyldiphenylmethyl)-6'-amino-5'-homothymidine, 50a

Compound **49a** is treated as described in Example 29 to give the title compound **50a**, as a white solid (93%).

Example 55

3'-O-{3-N-[(4-N-(isobutyryl)cytidine-1-yl)acetyl]-3-N-(carboxymethyl)-3-aminopropyl}-6'-N-(p-anisyldiphenylmethyl)-6'-amino-5'-homothymidine, 50b

Compound **49b** is treated as described in Example 30 to give the title compound **50b**, as a white solid (95%).

Example 56

3'-O-{3-N-[(thymine-1-yl)acetyl]-3-N-(pentafluorophenoxycarbonylmethyl)-3-aminopropyl}-6'-N-(p-anisyldiphenylmethyl)-6'-amino-5'-homothymidine, 51a

Compound **50a** is treated as described in Example 32 to give a solution of the title compound **51a**.

Example 57

3'-O-{3-N-[(4-N-(isobutyryl)cytidine-1-yl)acetyl]-3-N-(pentafluorophenoxycarbonylmethyl)-3-aminopropyl}-6'-N-(p-anisyldiphenylmethyl)-6'-amino-5'-homothymidine, 51b

Compound **50b** is treated as described in Example 33 to give a solution of the title compound **51b**.

Example 58

3'-O-{3-N-[(thymine-1-yl)acetyl]-3-N-(N-(3-N-(diethylamino) propyl)carboxamidomethyl)-3-aminopropyl)-6'-N-(p-anisyldiphenylmethyl)-6'-

amino-5'-homothymidine, 52a

A solution of compound **51a** (1 mmole) in DMF (5 ml) is treated with 3-N-(diethylamino)propylamine (1.5 mmole) at ambient temperature for 2h. The reaction mixture is evaporated *in vacuo* to dryness and the residue is chromatographed over a silica gel column (2.5x25 cm) using $CHCl_3$/28%NH_4OH (99.9/0.1) (gradient of MeOH from 0 to 9%) as eluent to give compound **52a**, (85%), as an oil.

Example 59

Oligonucleoside 53 (n=1)

A solution of compound **52** (1 mmole) in 80% AcOH 100 ml is kept overnight at ambient temperature. The reaction mixture is evaporated *in vacuo* to dryness and the residue is coevaporated with Py (3x50 ml) and anhydrous DMF (3x25 ml). The residue is dissolved in anhydrous DMF (5 ml) and solution of compound **51** (1.2 mmole) and DIPEA (1.1 mmole) in anhydrous DMF (5 ml) is added. The reaction mixture is kept at ambient temperature for 2h. The reaction mixture is evaporated *in vacuo* to dryness and the residue is chromatographed over a silica gel column (2.5x25 cm) using $CHCl_3$/28%NH_4OH (99.9/0.1) (gradient of MeOH from 0 to 10%) as eluent to give compound **53 (n=1)**, (89%).

Example 60

Oligonucleoside 53 (n=2)

Compound **53 (n=1)** is treated as described in Example 59 to give the title compound **53 (n=2)**, as a white solid (95%).

Example 61

Oligonucleoside 53 (n=3)

Compound **53 (n=2)** is treated as described in Example 59 to give the title compound **53 (n=3)**, as a white solid (94%).

Example 62

Oligonucleoside 53 (n=4)

Compound **53** (**n=3**) is treated as described in Example 59 to give the title compound **53** (**n=4**), as a white solid (90%).

Example 63

Oligonucleoside 53 (n=5)

Compound **53** (**n=4**) is treated as described in Example 59 to give the title compound **53** (**n=5**), as a white solid (91%).

Example 64

Oligonucleoside 54

A solution of compound **53** (1 mmole) in 80% AcOH 100 ml is kept overnight at ambient temperature. The reaction mixture is evaporated *in vacuo* to dryness and the residue is dissolved in water and extracted twice with ether. The water layer is evaporated *in vacuo* to dryness and the residue is lyophilized twice from water.

Example 65

Once phosphoramidites of modified nucleosides and oligonucleoside synthetic blocks have been prepared, they can be incorporated into oligonucleotide analogues, which are synthesized by a stantard solid phase approach, using automated nucleic acid synthesizer such as Applied Biosystems, Inc. Model 392. Standard phosphoramidite coupling chemistries (see, e.g. M.J. Gait, Oligonucleotide Synthesis. A practical approach., pp. 35-81) are used with these synthesizers to furnish desired oligonucleotides. The solution of tetraethylthiuram disulfide (Applied Biosystems, Inc.) can be used to provide phosphorthioate oligonucleotides.

Example 66

Hybridization Analysis

The macromolecules of the invention can be compared in their ability to bind to complementary nucleic acids by determining the melting temperature of a particular double-stranded (ds) or triple-stranded (ts) complex. Upon formation of dsDNA from two single strands, due to base stacking the extinction coefficient decreases (hypochromicity). Consequently, the denaturating of DNA can be followed by measuring changes in the absorbance, as a function of the melting temperature (T_m), the temperature where 50% of a duplex has disappeared to give single strands. The higher the T_m, the greater the strengh of the binding of the strands.

Duplexes are formed from single-stranded oligodeoxyribonucleotides and the macromolecules of the present invention. The macromolecules of the present invention are synthesized according the description or examples presented herein. Oligodeoxyribonucleotides are synthesized on solid phase with an Applied Biosystems, Inc. 392 DNA/RNA Synthesizer. The oligonucleotide species is purified as their dimethoxytrityl derivatives by reverse-phase chromatography using HPLC (Gilson). Typically 0.3-0.5 OD_{260} oligonucleotide analog is hybridized with 1 equivalent of the other strand and the absorbance (260 nm) hyperchromicity upon duplex to random coil transition is monitored using a Gilford Response II spectrophotometer. The buffers used are 10 mM in phosphate, 0.1 mM in EDTA and either 0.1 M or 1 M in NaCl. The following extinction coefficients are used dA: 15.4 ml/μmol·cm; dT 8.8; dG: 11.7 and dC: 7.3 for both regular oligonucleotides and oligonucleotide analogues. The melting curves are recorded in steps of 0.5 °C/min. The T_m is determined from the maximum of the 1st derivative of the plot A_{260} vs temperature. Data can be analyzed by the graphic

representation of $1/T_m$ vs $\ln[C_{oligo}]$, wherein C_{oligo} is the total oligonucleotide concentration. Thermodynamic parameters are determined from this analysis.

Nuclease Resistance

A. Evaluation of the resistance of macromolecules of the invention to serum and cytoplasmic nucleases.

The oligonucleotide analogues of the invention were evaluated for their stability in media containing various concentrations of fetal calf serum or adult human serum. Oligonucleotide analogs are incubated for various times, treated with protease K and then analyzed by reverse-phase or ion-exchange HPLC or by gel-electrophoresis on 20% polyacrylamide-urea denaturating gels and subsequent autoradiography. Based on the location of the modified linkage and the known length of the oligonucleotide it is possible to determine the effect on nuclease degradation depending on the particular modification of the linkage. For the cytoplasmic nucleases, an HL 60 cell line can be used.

Following the incubation, the oligonucleotide analogs are assessed for degradation as mentioned above for serum nucleolytic degradation. Autoradiography results are quantitated to compare the regular oligonucleotides and macromolecules of invention.

B. Evaluation of the resistance of macromolecules of the invention to specific endo- and exo-nucleases.

Evaluation of the resistance of oligonucleotide analogues of the invention to specific nucleases (i.e. endonucleases, 3', 5'-exo-, and 5', 3'-exonucleases) can be done to estimate precisely the effect of a particular linkage on stability in degradation conditions. The oligonucleotide analogs are incubated in defined reaction buffers specific for various selected nucleases, treated

with proteinase K and then analyzed by reverse-phase or ion-exchange HPLC, or by gel-electrophoresis on 20% polyacrylamide-urea denaturating gels and subsequent autoradiography.

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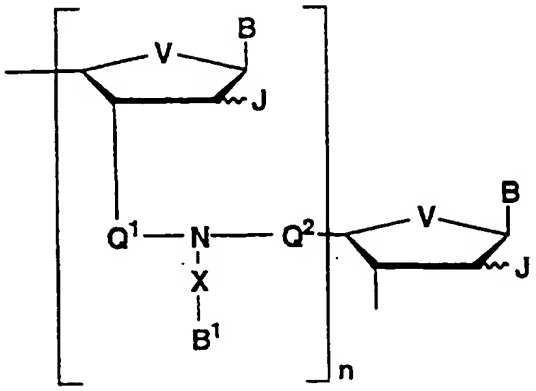
We claim:

1. A macromolecule, at least a portion of which is of the structure:



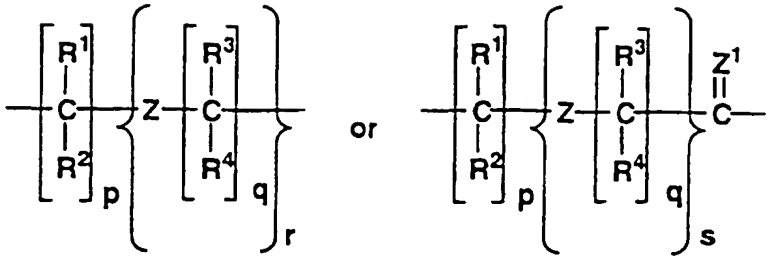
wherein

each B is independently hydrogen, hydroxy, a naturally occurring nucleobase, a non-naturally occurring nucleobase, a DNA intercalator, a covalent or non-covalent DNA-binding group, a heterocyclic moiety, or an aromatic moiety;

each B^1 is independently hydrogen, hydroxy, amino, mercapto, a naturally occurring nucleobase, a non-naturally occurring nucleobase, a DNA intercalator, a covalent or non-covalent DNA-binding group, a heterocyclic moiety, an aromatic moiety, a targeting group, a carrier, a reporter group, or a soluble or non-soluble polymer;

n is an integer from 1 to 50;

each X is independently a single bond, methylene, methylenecarbonyl, C_7-C_{12} aralkylene or substituted aralkylene, C_7-C_{12} aralkylenecarbonyl or substituted aralkylenecarbonyl, or a group of the formula:



wherein

each **Z** is independently a single bond, O, S, NR^6, $C(=O)NR^6$ or $C(=O)NR^6$;

each Z^1 is independently O, S, NR^5, methylene, or $C(CH_3)_2$;

each of **p**, **q**, **r**, and **s** is independently an integer from 0 to 20;

each of R^1, R^2, R^3 and R^4 is independently hydrogen; C_1-C_8 alkyl, which may be hydroxy-, or alkoxy-, or alkylthio-substituted; hydroxy; alkoxy; alkylthio; amino or halogen;

each of R^5 and R^6 is independently hydrogen; C_1-C_8 alkyl, which may be hydroxy-, or alkoxy-, or alkylthio-substituted; hydroxy; alkoxy; alkylthio; or amino;

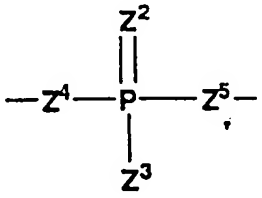
each of Q^1 or Q^2 independently comprises at least three atoms, at least one of which is carbon;

each **V** is independently oxygen, sulfur, NR^8 or methylene; and

each **J** is independently hydrogen, azido, halogen, $-OR^7$, $-R^7$ or $-NR^7R^8$, wherein each R^7 is independently $-NR^8R^9$ or R^8, wherein each of R^8 or R^9 is independently hydrogen, C_3-C_{10} branched alkyl or substituted alkyl, C_1-C_{10} unbranched alkyl or substituted alkyl, C_1-C_{10} unbranched oxaalkyl or substituted oxaalkyl, C_6-C_{10} aryl or substituted aryl, C_7-C_{12} aralkyl or substituted aralkyl, C_1-C_{10} unbranched aminoalkyl or substituted unbranched aminoalkyl; C_1-C_{10} unbranched aminooxaalkyl or substituted unbranched

aminooxaalkyl, C_3-C_{10} and N_1-N_4 branched (polyamino- or polyaza-)alkyl or substituted (polyamino- or polyaza-) alkyl, C_1-C_{10} and N_1-N_4 unbranched (polyamino- or polyaza-) alkyl or substituted (polyamino- or polyaza-)alkyl, C_1-C_{10} and N_1-N_4 unbranched (polyamino- or polyaza-)oxaalkyl or substituted unbranched (polyamino- or polyaza-)oxaalkyl, a natural or non-natural amino acid side chain radical, or a protecting group.

2. The macromolecule of claim 1, wherein each of Q^1 and Q^2 is independently at least one of the following: oxygen; sulfur; substituted carbon; carbonyl; thiocarbonyl; sulfone; sulfoxide; C_1-C_8 alkylene; C_2-C_8 alkenylene; C_2-C_8 alkynylene; C_1-C_8 oxaalkylene or thiaalkylene or azaalkylene each containing one or two different heteroatoms or hetroatoms of the same type; NR^7, $^+NR^8R^9$, $NR^7C(=O)-$, $-NR^7C(=S)-$, $-NR^7S(=O)-$, or $-NR^7S(=O)_2-$ in either orientation, wherein R^7, R^8 and R^9 are as specified above; X, wherein X has been specified above; or a group of:



wherein

each of Z^4 or Z^5 is independently a single bond, O, S, and NR^7, wherein R^7 has been specified above in claim 1;

each Z^3 independently comprises hydrogen, R^8, OR^7, SR^7, and NR^7R^8, wherein R^7 and R^8 have been specified above in claim 1;

each Z^2 independently comprises O, S, or NR^7, wherein R^7 has been specified above in claim 1.

3. The macromolecule of claim 2 wherein each Q^1 is independently $-O-CH_2-CH_2-CH_2-$, $-O-CH_2-C(=O)-NH-$,

$-NH-NH-C(=O)-CH_2-$, $-NH-N=CH-CH_2-$, $-NH-NH-CH_2-CH_2-$, $-O-NH-C(=O)-CH_2-$, $-O-N=CH-CH_2-$, $-O-NH-CH_2-CH_2-$, $-CH_2-NH-C(=O)-CH_2-$, $-NH-NH-C(=O)-NH-$, $-O-C(=O)-NH-CH_2-CH_2-$, $-O-P(=Z^2)Z^3-NH-CH_2-CH_2-$, or $-CH_2-NH-CH_2-CH_2-$, in either orientation; and each Q^2 is independently $-CH_2-O-CH_2-CH_2-CH_2-$, $-CH_2-O-CH_2-C(=O)-NH-$, $-CH_2-NH-NH-C(=O)-CH_2-$, $-CH_2-NH-N=CH-CH_2-$, $-CH_2-NH-NH-CH_2-CH_2-$, $-CH_2-O-NH-C(=O)-CH_2-$, $-CH_2-O-N=CH-CH_2-$, $-CH_2-O-NH-CH_2-CH_2-$, $-CH_2-NH-C(=O)-CH_2-$, $-CH_2-NH-NH-C(=O)-NH-$, $-O-C(=O)-NH-CH_2-CH_2-$, $-O-P(=Z^2)Z^3-NH-CH_2-CH_2-$, or $-CH_2-CH_2-NH-CH_2-CH_2-$, in either orientation.

4. The macromolecule of claim 3 wherein n=1.

5. The macromolecule of claim 2 wherein at least one of V is oxygen.

6. The macromolecule of claim 3 wherein at least one of V is oxygen.

7. The macromolecule of claim 4 wherein at least one of V is oxygen.

8. The macromolecule of claim 2 wherein at least one of J is H.

9. The macromolecule of claim 3 wherein at least one of J is H.

10. The macromolecule of claim 6 wherein at least one of J is H.

11. The macromolecule of claim 7 wherein at least one of J is H.

12. The macromolecule of claim 2 wherein at least one of B is a naturally or non-naturally occurring nucleobase.

13. The macromolecule of claim 3 wherein at least one of B is a naturally or non-naturally occurring nucleobase.

14. The macromolecule of claim 10 wherein at least one of B is naturally or non-naturally occurring nucleobase.

15. The macromolecule of claim 11 wherein at least

one of B is naturally or non-naturally occurring nucleobase.

16. The macromolecule of claim 12 wherein at least one of B^1 comprises a DNA intercalator, a covalent DNA-binding group, or a non-covalent DNA-binding group.

17. The macromolecule of claim 13 wherein at least one of B^1 comprises a DNA intercalator, a covalent DNA-binding group, or a non-covalent DNA-binding group.

18. The macromolecule of claim 14 wherein at least one of B^1 comprises a DNA intercalator, a covalent DNA-binding group, or a non-covalent DNA-binding group.

19. The macromolecule of claim 15 wherein at least one of B^1 comprises a DNA intercalator, a covalent DNA-binding group or a non-covalent DNA-binding group.

20. The macromolecule of claim 12 wherein at least one of B^1 is a naturally or non-naturally occurring nucleobase.

21. The macromolecule of claim 13 wherein at least one of B^1 is a naturally or non-naturally occurring nucleobase.

22. The macromolecule of claim 14 wherein at least one of B^1 is a naturally or non-naturally occurring nucleobase.

23. The macromolecule of claim 15 wherein at least one of B^1 is a naturally or non-naturally occurring nucleobase.

24. The macromolecule of claim 16 wherein at least one of X independently comprises C_1-C_{10} alkylene or substituted alkylene, C_1-C_{10} alkylenecarbonyl or substituted alkylenecarbonyl, polyamide, C_7-C_{12} aralkylene or substituted aralkylene, or C_7-C_{12} aralkylenecarbonyl or substituted aralkylenecarbonyl.

25. The macromolecule of claim 17 wherein at least one of X independently comprises C_1-C_{10} alkylene or

substituted alkylene, C_1-C_{10} alkylenecarbonyl or substituted alkylenecarbonyl, polyamide, C_7-C_{12} aralkylene or substituted aralkylene, or C_7-C_{12} aralkylenecarbonyl or substituted aralkylenecarbonyl.

26. The macromolecule of claim 18 wherein at least one of X independently comprises C_1-C_{10} alkylene or substituted alkylene, C_1-C_{10} alkylenecarbonyl or substituted alkylenecarbonyl, polyamide, C_7-C_{12} aralkylene or substituted aralkylene, or C_7-C_{12} aralkylenecarbonyl or substituted aralkylenecarbonyl.

27. The macromolecule of claim 19 wherein at least one of X independently comprises C_1-C_{10} alkylene or substituted alkylene, C_1-C_{10} alkylenecarbonyl or substituted alkylenecarbonyl, polyamide, C_7-C_{12} aralkylene or substituted aralkylene, or C_7-C_{12} aralkylenecarbonyl or substituted aralkylenecarbonyl.

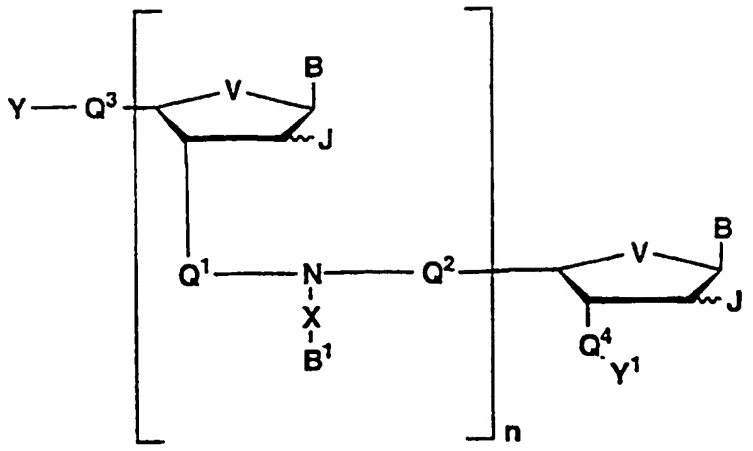
28. The macromolecule of claim 20 wherein at least one of X independently comprises C_1-C_4 alkylene or substituted alkylene, or C_1-C_4 alkylenecarbonyl or substituted alkylenecarbonyl.

29. The macromolecule of claim 21 wherein at least one of X independently comprises C_1-C_4 alkylene or substituted alkylene, or C_1-C_4 alkylenecarbonyl or substituted alkylenecarbonyl.

30. The macromolecule of claim 22 wherein at least one of X is methylenecarbonyl.

31. The macromolecule of claim 23 wherein at least one of X is methylenecarbonyl.

32. A compound having the formula:



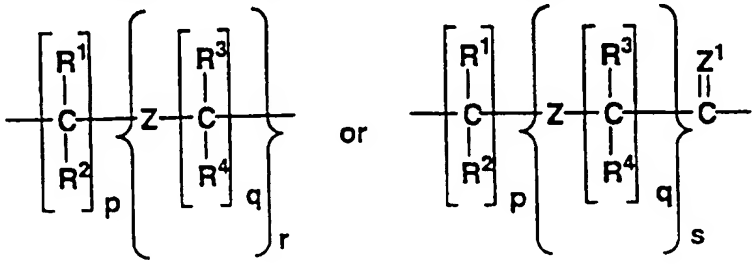
wherein

each B is independently a naturally occurring nucleobase, a non-naturally occurring nucleobase, a heterocyclic moiety, or an aromatic moiety, any of which optionally contains a protecting group;

each B^1 is independently hydrogen, hydroxy, amino, mercapto, a naturally occurring nucleobase, a non-naturally occurring nucleobase, a DNA intercalator, a covalent or non-covalent DNA-binding group, a heterocyclic moiety, or an aromatic moiety, any of which optionally contains a protecting group;

n is an integer from 1 to 50;

each X is independently an optionally protected group selected from a single bond, methylene group, methylenecarbonyl, C_7-C_{12} aralkylene or substituted aralkylene, C_7-C_{12} aralkylenecarbonyl or substituted aralkylenecarbonyl or a group of the formula:



wherein

each Z is independently a single bond, O, S, NR^6, $C(=O)NR^6$, $C(=S)NR^6$, $S(=O)NR^6$, or $S(=O)_2NR^6$;

each Z^1 is independently O, S, Se, NR^5, methylene, or $C(CH_3)_2$;

each of p, q, r, and s is independently an integer from 0 to 20;

each of R^1, R^2, R^3 and R^4 is independently hydrogen; C_1-C_8 alkyl, which may be hydroxy-, or alkoxy-, or alkylthio-substituted; hydroxy; alkoxy; alkylthio; amino or halogen;

each of R^5 and R^6 is independently hydrogen; C_1-C_8 alkyl, which may be hydroxy-, or alkoxy-, or alkylthio-substituted; hydroxy; alkoxy; alkylthio; or amino;

each of Q^1 or Q^2 independently comprises at least three atoms, at least one of which is carbon;

each V is independently oxygen, sulfur, NR^8 or methylene, wherein R^8 is independently hydrogen, C_3-C_{10} branched alkyl or substituted alkyl, C_1-C_{10} unbranched alkyl or substituted alkyl, C_1-C_{10} unbranched oxaalkyl or substituted oxaalkyl, C_6-C_{10} aryl or substituted aryl, C_7-C_{12} aralkyl or substituted aralkyl, C_1-C_{10} unbranched aminoalkyl or substituted unbranched aminoalkyl; C_1-C_{10} unbranched aminooxaalkyl or substituted unbranched aminooxaalkyl, C_3-C_{10} and N_1-N_4 branched (polyamino- or polyaza-)alkyl or substituted (polyamino- or polyaza-)alkyl, C_1-C_{10} and N_1-N_4 unbranched (polyamino- or polyaza-)alkyl or substituted (polyamino- or polyaza-)alkyl, C_1-C_{10} and N_1-N_4 unbranched (polyamino- or polyaza-)oxaalkyl or substituted unbranched (polyamino- or polyaza-)oxaalkyl, a natural or non-natural amino acid side chain radical, or a protecting group;

each J is independently, hydrogen, OR^7, halogen, azide or R^7, any of which is optionally protected, wherein each R^7 is independently $-NR^8R^9$ or R^8, wherein R^9 is

independently hydrogen, C_3-C_{10} branched alkyl or substituted alkyl, C_1-C_{10} unbranched alkyl or substituted alkyl, C_1-C_{10} unbranched oxaalkyl or substituted oxaalkyl, C_6-C_{10} aryl or substituted aryl, C_7-C_{12} aralkyl or substituted aralkyl, C_1-C_{10} unbranched aminoalkyl or substituted unbranched aminoalkyl; C_1-C_{10} unbranched aminooxaalkyl or substituted unbranched aminooxaalkyl, C_3-C_{10} and N_1-N_4 branched (polyamino- or polyaza-)alkyl or substituted (polyamino- or polyaza-) alkyl, C_1-C_{10} and N_1-N_4 unbranched (polyamino- or polyaza-) alkyl or substituted (polyamino- or polyaza-) alkyl, C_1-C_{10} and N_1-N_4 unbranched (polyamino- or polyaza-) oxaalkyl or substituted unbranched (polyamino- or polyaza-) oxaalkyl, a natural or non-natural amino acid side chain radical, or a protecting group;

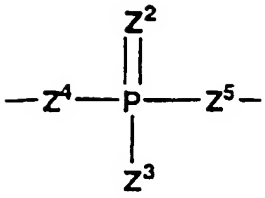
each Q^3 is independently -OX-, -SX- or -NR^8X-, any of which is optionally protected;

each Q^4 is independently oxygen, sulfur or NR^8, any of which is optionally protected;

Y is a protecting group;

Y^1 is a spacer group linked to a solid support.

33. The compound of claim 32, wherein each of Q^1 and Q^2 independently contains at least one of the following groups, any of which is optionally protected: oxygen; sulfur; substituted carbon; carbonyl; thiocarbonyl; sulfone; sulfoxide; C_1-C_8 alkylene; C_2-C_8 alkenylene; C_2-C_8 alkynylene; C_1-C_8 oxaalkylene or thiaalkylene or azaalkylene each containing one or two different heteroatoms or hetroatoms of the same type; NR^7, $^+NR^8R^9$, $NR^7C(=O)$-, -$NR^7C(=S)$-, -$NR^7S(=O)$-, or -$NR^7S(=O)_2$- in either orientation, wherein R^7, R^8 and R^9 have been specified above; or X, wherein X has been specified above; or a group of:



wherein

each of Z^1 or Z^4 is independently a single bond, O, S, or NR^7;

each Z^3 is independently hydrogen, R^8, OR^7, SR^7, or NR^7R^8; and

each Z^2 is independently O, S, or NR^7.

34. The compound of claim **33**, wherein each Q^1 comprises one of the following, any of which optionally contains a protecting group: $-O-CH_2-CH_2-CH_2-$, $-O-CH_2-C(=O)-NH-$, $-NH-NH-C(=O)-CH_2-$, $-NH-N=CH-CH_2-$, $-NH-NH-CH_2-CH_2-$, $-O-NH-C(=O)-CH_2-$, $-O-N=CH-CH_2-$, $-O-NH-CH_2-CH_2-$, $-CH_2-NH-C(=O)-CH_2-$, $-NH-NH-C(=O)-NH-$, $-O-C(=O)-NH-CH_2-CH_2-$, $-O-P(=Z^2)Z^3-NH-CH_2-CH_2-$, or $-CH_2-NH-CH_2-CH_2-$, in either orientation; and each Q^2 comprises the following, any of which optionally contains a protecting group: $-CH_2-O-CH_2-CH_2-CH_2-$, $-CH_2-O-CH_2-C(=O)-NH-$, $-CH_2-NH-NH-C(=O)-CH_2-$, $-CH_2-NH-N=CH-CH_2-$, $-CH_2-NH-NH-CH_2-CH_2-$, $-CH_2-O-NH-C(=O)-CH_2-$, $-CH_2-O-N=CH-CH_2-$, $-CH_2-O-NH-CH_2-CH_2-$, $-CH_2-NH-C(=O)-CH_2-$, $-CH_2-NH-NH-C(=O)-NH-$, or $-CH_2-CH_2-NH-CH_2-CH_2-$, in either orientation.

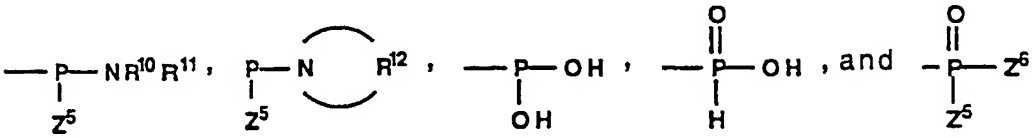
35. The compound of claim **33** wherein **Y** is p-anisyldiphenylmethyl, di-p-anisylphenylmethyl, or pixyl.

36. The compound of claim **34** wherein **Y** is p-anisyldiphenylmethyl, di-p-anisylphenylmethyl, or pixyl.

37. The compound of claim **35** wherein the spacer group comprises carbonyl, ester, carbamate, urethane, hydrazide, C_1-C_{14} alkylene or modified alkylene, C_6-C_{14} aralkylene or

modified aralkylene, C_6-C_{14} alkylarene or modified alkylarene, C_1-C_{100} oxaalkylene or thiaalkylene or azaalkylene each containing from one to fifty different heteroatoms or hetroatoms of the same type, where aza groups are, optionally, protected by amino protecting groups, C_1-C_{14} alkylenecarbonyl or alkylenethiocarbonyl or alkylenesulfone or alkylenesulfoxide, C_1-C_{100} oxaalkylenecarbonyl or thiaalkylenecarbonyl or azaalkylenecarbonyl (or their thiocarbonyl or sulfone or sulfoxide analogs) each containing from one to fifty different heteroatoms or hetroatoms of the same type, where aza groups are, optionally, protected by amino protecting groups;

or a group of the formula:



and salts thereof, wherein

each of R^{10} or R^{11} is independently selected from the group consisting of C_3-C_{10} branched alkyl, C_1-C_{10} unbranched alkyl or oxaalkyl, C_6-C_{10} aryl, C_7-C_{12} aralkyl;

R^{12} is C_2-C_8 alkylene, C_2-C_8 alkenylene or -C_2-C_8 oxaalkylene, comprising one or two heteroatoms;

Z^5 is a phosphate protecting group;

Z^6 is halogen, imidazol-1-yl, tetrazol-1-yl, 1,2,4-triazol-1-yl and 1-hydroxy-benzotriazol-O-yl.

38. The compound of claim 35 wherein Y^1 is -HP(=O)OH or salts thereof; $-P(OCH_2CH_2CN)(N(i\text{-}Pr)_2)$; or $-P(OCH_3)(N(i\text{-}Pr)_2)$.

39. The compound of claim 36 wherein Y^1 is -HP(=O)OH or salts thereof; $-P(OCH_2CH_2CN)(N(i\text{-}Pr)_2)$; or $-P(OCH_3)(N(i\text{-}Pr)_2)$.

40. The compound of claim 37 wherein n=1.

41. The compound of claim 38 wherein n=1.

42. The compound of claim 39 wherein n=1.

43. The compound of claim 33 wherein at least one of V is oxygen.

44. The compound of claim 37 wherein at least one of V is oxygen.

45. The compound of claim 39 wherein at least one of V is oxygen.

46. The compound of claim 41 wherein at least one of V is oxygen.

47. The compound of claim 42 wherein at least one of V is oxygen.

48. The compound of claim 33 wherein at least one of Q^3 or Q^4 is oxygen.

49. The compound of claim 36 wherein at least one of Q^3 or Q^4 is oxygen.

50. The compound of claim 44 wherein at least one of Q^3 or Q^4 is oxygen.

51. The compound of claim 45 wherein at least one of Q^3 or Q^4 is oxygen.

52. The compound of claim 46 wherein at least one of Q^3 or Q^4 is oxygen.

53. The compound of claim 33 wherein each J is independently hydrogen, OR^7, or fluorine, wherein any of OR^7 optionally contains a protecting group.

54. The compound of claim 36 wherein at least one of J is hydrogen.

55. The compound of claim 37 wherein at least one of J is hydrogen.

56. The compound of claim 43 wherein at least one of J is hydrogen.

57. The compound of claim 45 wherein at least one of J is hydrogen.

58. The compound of claim 47 wherein at least one of

J is hydrogen.

59. The compound of claim 51 wherein at least one of J is hydrogen.

60. The compound of claim 52 wherein at least one of J is hydrogen.

61. The compound of claim 33 wherein at least one B is a naturally or non-naturally occurring nucleobase, any of which optionally contains a protecting group.

62. The compound of claim 36 wherein at least one B is a naturally or non-naturally occurring nucleobase, any of which optionally contains a protecting group.

63. The compound of claim 44 wherein at least one B is a naturally or non-naturally occurring nucleobase, any of which optionally contains a protecting group.

64. The compound of claim 45 wherein at least one B is a naturally or non-naturally occurring nucleobase, any of which optionally contains a protecting group.

65. The compound of claim 47 wherein at least one B is a naturally or non-naturally occurring nucleobase, any of which optionally contains a protecting group.

66. The compound of claim 59 wherein at least one B is a naturally or non-naturally occurring nucleobase, any of which optionally contains a protecting group.

67. The compound of claim 60 wherein at least one B is a naturally or non-naturally occurring nucleobase, any of which optionally contains a protecting group.

68. The compound of claim 61 wherein each B^1 is a naturally or non-naturally occurring nucleobase, any of which optionally contains a protecting group.

69. The compound of claim 62 wherein each B^1 is a naturally or non-naturally occurring nucleobase, any of which optionally contains a protecting group.

70. The compound of claim 63 wherein each B^1 is a naturally or non-naturally occurring nucleobase, any of which optionally contains a protecting group.

71. The compound of claim 64 wherein each B^1 is a

naturally or non-naturally occurring nucleobase, any of which optionally contains a protecting group.

72. The compound of claim 65 wherein each B^1 is a naturally or non-naturally occurring nucleobase, any of which optionally contains a protecting group.

73. The compound of claim 66 wherein each B^1 is a naturally or non-naturally occurring nucleobase, any of which optionally contains a protecting group.

74. The compound of claim 67 wherein each B^1 is a naturally or non-naturally occurring nucleobase, any of which optionally contains a protecting group.

75. The compound of claim 68 wherein at least one X is C_1-C_4 alkylene or substituted alkylene, or C_1-C_4 alkylenecarbonyl or substituted alkylenecarbonyl, any of which optionally contains a protecting group.

76. The compound of claim 69 wherein at least one X is C_1-C_4 alkylene or substituted alkylene, or C_1-C_4 alkylenecarbonyl or substituted alkylenecarbonyl, any of which optionally contains a protecting group.

77. The compound of claim 70 wherein at least one X is C_1-C_4 alkylene or substituted alkylene, or C_1-C_4 alkylenecarbonyl or substituted alkylenecarbonyl, any of which optionally contains a protecting group.

78. The compound of claim 71 wherein at least one X is C_1-C_4 alkylene or substituted alkylene, or C_1-C_4 alkylenecarbonyl or substituted alkylenecarbonyl, any of which optionally contains a protecting group.

79. The compound of claim 72 wherein at least one X is methylenecarbonyl.

80. The compound of claim 73 wherein at least one X is methylenecarbonyl.

81. The compound of claim 74 wherein at least one X is methylenecarbonyl.

82. The compound of claim 61 wherein each B^1 is independently hydrogen, amino, mercapto, a DNA

intercalator, a DNA-binding group, a polymer group, or a targeting group, any of which optionally contains a protecting group.

83. The compound of claim 62 wherein each B^1 is independently hydrogen, amino, mercapto, a DNA intercalator, a DNA-binding group, a polymer group, or a targeting group, any of which optionally contains a protecting group.

84. The compound of claim 63 wherein each B^1 is independently hydrogen, amino, mercapto, a DNA intercalator, a DNA-binding group, a polymer group, or a targeting group, any of which optionally contains a protecting group.

85. The compound of claim 64 wherein each B^1 is independently hydrogen, amino, mercapto, a DNA intercalator, a DNA-binding group, a polymer group, or a targeting group, any of which optionally contains a protecting group.

86. The compound of claim 65 wherein each B^1 is independently hydrogen, amino, mercapto, a DNA intercalator, a DNA-binding group, a polymer group, or a targeting group, any of which optionally contains a protecting group.

87. The compound of claim 66 wherein each B^1 is independently hydrogen, amino, mercapto, a DNA intercalator, a DNA-binding group, a polymer group, or a targeting group, any of which optionally contains a protecting group.

88. The compound of claim 67 wherein each B^1 is independently hydrogen, amino, mercapto, a DNA intercalator, a DNA-binding group, a polymer group, or a targeting group, any of which optionally contains a protecting group.

89. The compound of claim 82 wherein at least one X is a single bond, C_1-C_{10} alkylene or substituted alkylene, C_1-C_{10} alkylenecarbonyl or substituted alkylenecarbonyl, C_1-

C_{10} oxaalkylene or substituted oxaalkylene, C_1-C_{10} oxaalkylenecarbonyl or substituted oxaalkylenecarbonyl, polyamide, C_7-C_{12} aralkylene or substituted aralkylene, or C_7-C_{12} aralkylenecarbonyl or substituted aralkylenecarbonyl, any of which optionally contains a protecting group.

90. The compound of claim **83** wherein at least one **X** is a single bond, C_1-C_{10} alkylene or substituted alkylene, C_1-C_{10} alkylenecarbonyl or substituted alkylenecarbonyl, C_1-C_{10} oxaalkylene or substituted oxaalkylene, C_1-C_{10} oxaalkylenecarbonyl or substituted oxaalkylenecarbonyl, polyamide, C_7-C_{12} aralkylene or substituted aralkylene, or C_7-C_{12} aralkylenecarbonyl or substituted aralkylenecarbonyl, any of which optionally contains a protecting group.

91. The compound of claim **84** wherein at least one **X** is a single bond, C_1-C_{10} alkylene or substituted alkylene, C_1-C_{10} alkylenecarbonyl or substituted alkylenecarbonyl, C_1-C_{10} oxaalkylene or substituted oxaalkylene, C_1-C_{10} oxaalkylenecarbonyl or substituted oxaalkylenecarbonyl, polyamide, C_7-C_{12} aralkylene or substituted aralkylene, or C_7-C_{12} aralkylenecarbonyl or substituted aralkylenecarbonyl, any of which optionally contains a protecting group.

92. The compound of claim **85** wherein at least one **X** is a single bond, C_1-C_{10} alkylene or substituted alkylene, C_1-C_{10} alkylenecarbonyl or substituted alkylenecarbonyl, C_1-C_{10} oxaalkylene or substituted oxaalkylene, C_1-C_{10} oxaalkylenecarbonyl or substituted oxaalkylenecarbonyl, polyamide, C_7-C_{12} aralkylene or substituted aralkylene, or C_7-C_{12} aralkylenecarbonyl or substituted aralkylenecarbonyl, any of which optionally contains a protecting group.

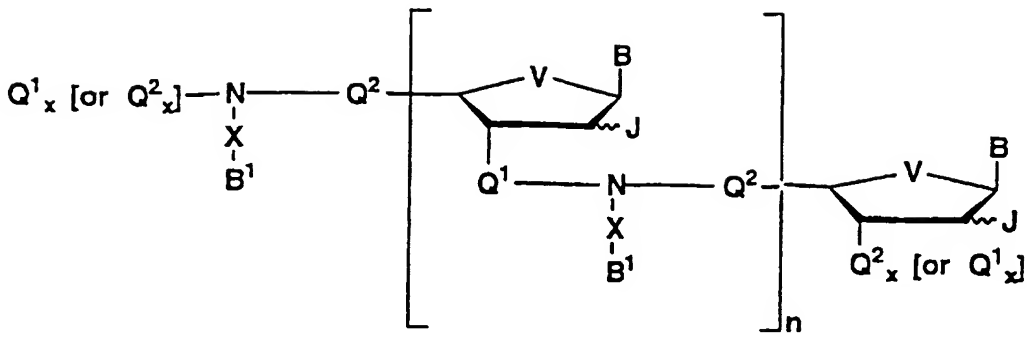
93. The compound of claim **86** wherein at least one **X** is a single bond, C_1-C_{10} alkylene or substituted alkylene, C_1-C_{10} alkylenecarbonyl or substituted alkylenecarbonyl, C_1-

C_{10} oxaalkylene or substituted oxaalkylene, C_1-C_{10} oxaalkylenecarbonyl or substituted oxaalkylenecarbonyl, polyamide, C_7-C_{12} aralkylene or substituted aralkylene, or C_7-C_{12} aralkylenecarbonyl or substituted aralkylenecarbonyl, any of which optionally contains a protecting group.

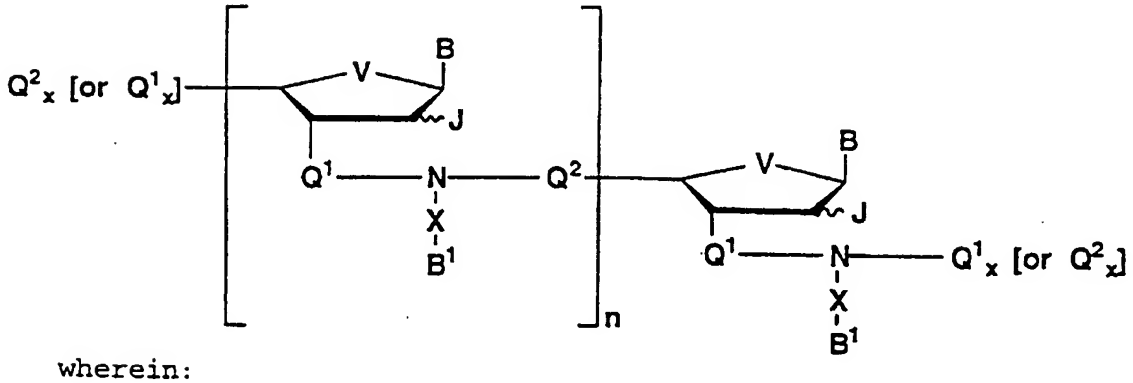
94. The compound of claim **87** wherein at least one **X** is a single bond, C_1-C_{10} alkylene or substituted alkylene, C_1-C_{10} alkylenecarbonyl or substituted alkylenecarbonyl, C_1-C_{10} oxaalkylene or substituted oxaalkylene, C_1-C_{10} oxaalkylenecarbonyl or substituted oxaalkylenecarbonyl, polyamide, C_7-C_{12} aralkylene or substituted aralkylene, or C_7-C_{12} aralkylenecarbonyl or substituted aralkylenecarbonyl, any of which optionally contains a protecting group.

95. The compound of claim **88** wherein at least one **X** is a single bond, C_1-C_{10} alkylene or substituted alkylene, C_1-C_{10} alkylenecarbonyl or substituted alkylenecarbonyl, C_1-C_{10} oxaalkylene or substituted oxaalkylene, C_1-C_{10} oxaalkylenecarbonyl or substituted oxaalkylenecarbonyl, polyamide, C_7-C_{12} aralkylene or substituted aralkylene, or C_7-C_{12} aralkylenecarbonyl or substituted aralkylenecarbonyl, any of which optionally contains a protecting group.

96. A compound represented by the formula:



or



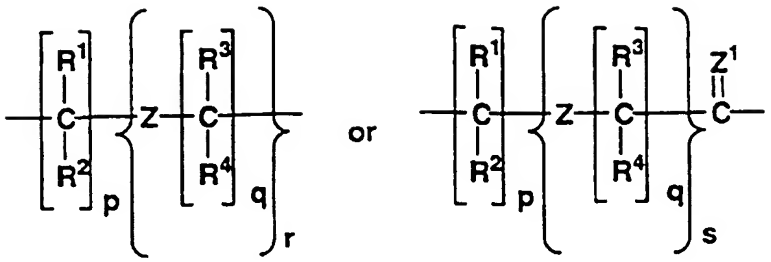
wherein:

each **B** is independently a naturally occurring nucleobase, a non-naturally occurring nucleobase, a heterocyclic moiety, or an aromatic moiety, any of which optionally contains a protecting group;

each B^1 is independently hydrogen, hydroxy, amino, mercapto, a naturally occurring nucleobase, a non-naturally occurring nucleobase, a DNA intercalator, a covalent or non-covalent DNA-binding group, a heterocyclic moiety, or an aromatic moiety, any of which optionally contains a protecting group;

n is an integer from 1 to 50;

each **X** is independently one of the following optionally protected groups: a single bond, methylene, methylenecarbonyl, C_7-C_{12} aralkylene or substituted aralkylene, C_7-C_{12} aralkylenecarbonyl or substituted aralkylenecarbonyl or a group of formula:



wherein

each **Z** is independently a single bond, O, S, NR^6, $C(=O)NR^6$, $C(=S)NR^6$, $S(=O)NR^6$, or $S(=O)_2NR^6$;

each Z^1 is independently O, S, Se, NR^5, methylene, or $C(CH_3)_2$;

each of **p**, **q**, **r** and **s** is independently an integer from 0 to 20;

each of R^1, R^2, R^3 and R^4 is independently hydrogen; C_1-C_8 alkyl, which may be hydroxy-, or alkoxy-, or alkylthio-substituted; hydroxy; alkoxy; alkylthio; amino or halogen;

each of R^5 and R^6 is independently hydrogen; C_1-C_8 alkyl, which may be hydroxy-, or alkoxy-, or alkylthio-substituted; hydroxy; alkoxy; alkylthio; or amino;

each of Q^1 or Q^2 comprises at least three atoms, at least one of which is carbon;

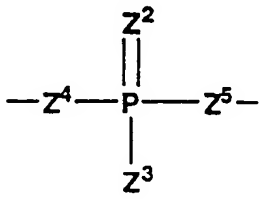
each **V** is independently oxygen, sulfur, NR^8 or methylene;

each **J** is independently one of the following optionally protected groups: hydrogen, OR^7, halogen, azide or R^7, wherein each R^7 is independently $-NR^8R^9$ or R^8, wherein each of R^8 or R^9 is independently hydrogen, C_3-C_{10} branched alkyl or substituted alkyl, C_1-C_{10} unbranched alkyl or substituted alkyl, C_1-C_{10} unbranched oxaalkyl or substituted oxaalkyl, C_6-C_{10} aryl or substituted aryl, C_7-C_{12} aralkyl or substituted aralkyl, C_1-C_{10} unbranched aminoalkyl

or substituted unbranched aminoalkyl; C_1-C_{10} unbranched aminooxaalkyl or substituted unbranched aminooxaalkyl, C_3-C_{10} and N_1-N_4 branched (polyamino- or polyaza-)alkyl or substituted (polyamino- or polyaza-)alkyl, C_1-C_{10} and N_1-N_4 unbranched (polyamino- or polyaza-)alkyl or substituted (polyamino- or polyaza-)alkyl, C_1-C_{10} and N_1-N_4 unbranched (polyamino- or polyaza-)oxaalkyl or substituted unbranched (polyamino- or polyaza-)oxaalkyl, a natural or non-natural amino acid side chain radical, or a protecting group;

each of Q^1_x and Q^2_x, comprising at least one atom, is independently selected from optionally protected or activated fragments of Q^1 or Q^2.

97. The compound of claim 96 wherein each of Q^1 and Q^2 independently contains at least one of the following groups, any of which is optionally protected: oxygen; sulfur; substituted carbon; carbonyl; thiocarbonyl; sulfone; sulfoxide; or C_1-C_8 alkylene; C_2-C_8 alkenylene; C_2-C_8 alkynylene; C_1-C_8 oxaalkylene or thiaalkylene or azaalkylene each containing one or two different heteroatoms or hetroatoms of the same type; NR^7, $^+NR^8R^9$, $NR^7C(=O)$-, $-NR^7C(=S)$-, $-NR^7S(=O)$-, or $-NR^7S(=O)_2$- in either orientation, wherein R^7, R^8 and R^9 have been specified above; or X, wherein X has been specified above; or a group of:



wherein

each of Z^4 or Z^5 is independently a single bond, O, S, or NR^7;

each Z^3 is independently hydrogen, R^8, OR^7, SR^7, or

NR^7R^8; and

each Z^2 is independently O, S, or NR^7.

98. The compound of claim 97 wherein each Q^1, any of which optionally contains a protecting group, independently comprises $-O-CH_2-CH_2-CH_2-$, $-O-CH_2-C(=O)-NH-$, $-NH-NH-C(=O)-CH_2-$, $-NH-N=CH-CH_2-$, $-NH-NH-CH_2-CH_2-$, $-O-NH-C(=O)-CH_2-$, $-O-N=CH-CH_2-$, $-O-NH-CH_2-CH_2-$, $-CH_2-NH-C(=O)-CH_2-$, $-NH-NH-C(=O)-NH-$, $-O-C(=O)-NH-CH_2-CH_2-$, $-O-P(=Z^2)Z^3-NH-CH_2-CH_2-$, or $-CH_2-NH-CH_2-CH_2-$, in either orientation; and

each Q^2, any of which optionally contains a protecting group, independently comprises Q^1, $-CH_2-O-CH_2-CH_2-CH_2-$, $-CH_2-O-CH_2-C(=O)-NH-$, $-CH_2-NH-NH-C(=O)-CH_2-$, $-CH_2-NH-N=CH-CH_2-$, $-CH_2-NH-NH-CH_2-CH_2-$, $-CH_2-O-NH-C(=O)-CH_2-$, $-CH_2-O-N=CH-CH_2-$, $-CH_2-O-NH-CH_2-CH_2-$, $-CH_2-NH-C(=O)-CH_2-$, $-CH_2-NH-NH-C(=O)-NH-$, or $-CH_2-CH_2-NH-CH_2-CH_2-$, in either orientation.

99. The compound of claim 97 wherein n=1.

100. The compound of claim 98 wherein n=1.

101. The compound of claim 98, wherein each of Q^1_x and Q^2_x contains from one to three atoms, any of which optionally contains a protecting group.

102. The compound of claim 100, wherein each of Q^1_x and Q^2_x contains from one to three atoms, any of which optionally contains a protecting group.

103. The compound of claim 101 wherein each Q^1_x is independently $Y^2-NH-CH_2-$, $Y^2-NH-O-$, $Y^2-NH-NH-$, Y^2-NH-, $Y^2-NH-CH_2-CH_2-$, $Y^2-NH-NH-CH_2-$, $Y^2-NH-C(=O)-CH_2-$, $Y^2-NH-C(=O)-NH-$, or $Y^2-NH-NH-C(=O)-$, wherein Y^2 is a protecting group or hydrogen; and

each Q^2_x is independently E-CH_2-CH_2-, E-CH_2-, E-NH-, E-O-, wherein E is independently halogen, aldehyde, acetal, -S(=O)$_2R^8$; or -COR^{13}, wherein R^{13} is hydroxyl or an activated group.

104. The compound of claim 102 wherein

each Q^1_x is independently Y^2-NH-CH_2-, Y^2-NH-O-, Y^2-NH-NH-, Y^2-NH-, Y^2-NH-CH_2-CH_2-, Y^2-NH-NH-CH_2-, Y^2-NH-C(=O)-CH_2-, Y^2-NH-C(=O)-NH-, or Y^2-NH-NH-C(=O)-, wherein Y^2 is a protecting group or hydrogen; and

each Q^2_x is independently E-CH_2-CH_2-, E-CH_2-, E-NH-, E-O-, wherein E is independently halogen, aldehyde, acetal, -S(=O)$_2R^8$; or -COR^{13}, wherein R^{13} is hydroxyl or an activated group.

105. The compound of claim 103 wherein Y^2 is p-anisyldiphenylmethyl, di-p-anisylphenylmethyl, pixyl, fluorenylmethoxycarbonyl, or trifluoroacetyl; and R^{13} is hydrogen, pentafluorophenyl, tetrafluorophenyl, p-nitrophenyl, or N-succinimidyl.

106. The compound of claim 104 wherein Y^2 is p-anisyldiphenylmethyl, di-p-anisylphenylmethyl, pixyl, fluorenylmethoxycarbonyl, or trifluoroacetyl; and R^{13} is hydrogen, pentafluorophenyl, tetrafluorophenyl, p-nitrophenyl, or N-succinimidyl.

107. The compound of claim 99 wherein at least one of V is oxygen.

108. The compound of claim 101 wherein at least one of V is oxygen.

109. The compound of claim 104 wherein at least one of V is oxygen.

110. The compound of claim 106 wherein at least one of V is oxygen.

111. The compound of claim 97 wherein at least one of J is hydrogen.

112. The compound of claim 101 wherein at least one of J is hydrogen.

113. The compound of claim 104 wherein at least one of J is hydrogen.

114. The compound of claim 110 wherein at least one of J is hydrogen.

115. The compound of claim 97 wherein at least one B is a naturally or non-naturally occurring nucleobase, any of which optionally contains a protecting group.

116. The compound of claim 101 wherein at least one B is a naturally or non-naturally occurring nucleobase, any of which optionally contains a protecting group.

117. The compound of claim 113 wherein at least one B is a naturally or non-naturally occurring nucleobase, any of which optionally contains a protecting group.

118. The compound of claim 114 wherein at least one B is a naturally or non-naturally occurring nucleobase, any of which optionally contains a protecting group.

119. The compound of claim 115 wherein each B^1 is a naturally or non-naturally occurring nucleobase, any of which optionally contains a protecting group.

120. The compound of claim 116 wherein each B^1 is a naturally or non-naturally occurring nucleobase, any of which optionally contains a protecting group.

121. The compound of claim 117 wherein each B^1 is a naturally or non-naturally occurring nucleobase, any of which optionally contains a protecting group.

122. The compound of claim 118 wherein each B^1 is a naturally or non-naturally occurring nucleobase, any of which optionally contains a protecting group.

123. The compound of claim 119 wherein at least one X is C_1-C_4 alkylene or substituted alkylene, or C_1-C_4 alkylenecarbonyl or substituted alkylenecarbonyl, any of which optionally contains a protecting group.

124. The compound of claim 120 wherein at least one X is C_1-C_4 alkylene or substituted alkylene, or C_1-C_4

alkylenecarbonyl or substituted alkylenecarbonyl, any of which optionally contains a protecting group.

125. The compound of claim 121 wherein at least one X is methylenecarbonyl.

126. The compound of claim 122 wherein at least one X is methylenecarbonyl.

127. The compound of claim 115 wherein each B^1 is independently hydrogen, amino, mercapto, a DNA intercalator, a DNA-binding group, a polymer group, or a targeting group, any of which optionally contains a protecting group.

128. The compound of claim 116 wherein each B^1 is independently hydrogen, amino, mercapto, a DNA intercalator, a DNA-binding group, a polymer group, or a targeting group, any of which optionally contains a protecting group.

129. The compound of claim 117 wherein each B^1 is independently hydrogen, amino, mercapto, a DNA intercalator, a DNA-binding group, a polymer group, or a targeting group, any of which optionally contains a protecting group.

130. The compound of claim 118 wherein each B^1 is independently hydrogen, amino, mercapto, a DNA intercalator, a DNA-binding group, a polymer group, or a targeting group, any of which optionally contains a protecting group.

131. The compound of claim 127 wherein at least one X is a single bond, C_1-C_{10} alkylene or substituted alkylene, C_1-C_{10} alkylenecarbonyl or substituted alkylenecarbonyl, C_1-C_{10} oxaalkylene or substituted oxaalkylene, C_1-C_{10} oxaalkylenecarbonyl or substituted oxaalkylenecarbonyl, polyamide, C_7-C_{12} aralkylene or substituted aralkylene, or C_7-C_{12} aralkylenecarbonyl or substituted aralkylenecarbonyl, any of which optionally contains a protecting group.

132. The compound of claim 128 wherein at least one X

is a single bond, C_1-C_{10} alkylene or substituted alkylene, C_1-C_{10} alkylenecarbonyl or substituted alkylenecarbonyl, C_1-C_{10} oxaalkylene or substituted oxaalkylene, C_1-C_{10} oxaalkylenecarbonyl or substituted oxaalkylenecarbonyl, polyamide, C_7-C_{12} aralkylene or substituted aralkylene, or C_7-C_{12} aralkylenecarbonyl or substituted aralkylenecarbonyl, any of which optionally contains a protecting group.

133. The compound of claim 129 wherein at least one X is a single bond, C_1-C_{10} alkylene or substituted alkylene, C_1-C_{10} alkylenecarbonyl or substituted alkylenecarbonyl, C_1-C_{10} oxaalkylene or substituted oxaalkylene, C_1-C_{10} oxaalkylenecarbonyl or substituted oxaalkylenecarbonyl, polyamide, C_7-C_{12} aralkylene or substituted aralkylene, or C_7-C_{12} aralkylenecarbonyl or substituted aralkylenecarbonyl, any of which optionally contains a protecting group.

134. The compound of claim 130 wherein at least one X is a single bond, C_1-C_{10} alkylene or substituted alkylene, C_1-C_{10} alkylenecarbonyl or substituted alkylenecarbonyl, C_1-C_{10} oxaalkylene or substituted oxaalkylene, C_1-C_{10} oxaalkylenecarbonyl or substituted oxaalkylenecarbonyl, polyamide, C_7-C_{12} aralkylene or substituted aralkylene, or C_7-C_{12} aralkylenecarbonyl or substituted aralkylenecarbonyl, any of which optionally contains a protecting group.

135. A pharmaceutical composition comprising an effective amount of the macromolecule of claim 1 and pharmaceutically acceptable carrier.

136. A pharmaceutical composition comprising an effective amount of the macromolecule of claim 6 and a pharmaceutically acceptable carrier.

137. A pharmaceutical composition comprising an effective amount of the macromolecule of claim 11 and a pharmaceutically acceptable carrier.

138. A pharmaceutical composition comprising an

effective amount of the macromolecule of claim 16 and a pharmaceutically acceptable carrier.

139. A pharmaceutical composition comprising an effective amount of the macromolecule of claim 19 and a pharmaceutically acceptable carrier.

140. A pharmaceutical composition comprising an effective amount of the macromolecule of claim 20 and a pharmaceutically acceptable carrier.

141. A pharmaceutical composition comprising an effective amount of the macromolecule of claim 23 and a pharmaceutically acceptable carrier.

142. A method for the treatment of a disease caused by a pathogenic organism, which comprises administering to a host organism in need of such treatment an effective amount of a compound according to claim 1.

143. A method for the treatment of a disease caused by a pathogenic organism, which comprises administering to a host organism in need of such treatment an effective amount of a compound according to claim 6.

144. A method for the treatment of a disease caused by a pathogenic organism, which comprises administering to a host organism in need of such treatment an effective amount of a compound according to claim 11.

145. A method for the treatment of a tumor, which comprises administering to an organism in need of such treatment an effective amount of a compound according to claim 1.

146. A method for the treatment of a tumor, which comprises administering to an organism in need of such treatment an effective amount of a compound according to claim 6.

147. A method for the treatment of a tumor, which comprises administering to an organism in need of such treatment an effective amount of a compound according to claim 11.

148. A method for the treatment of a disease caused by a pathogenic organism, which comprises administering to

a host organism in need of such treatment an effective amount of a pharmaceutical composition according to claim **135**.

149. A method for the treatment of a disease caused by a pathogenic organism, which comprises administering to a host organism in need of such treatment an effective amount of a pharmaceutical composition according to claim **136**.

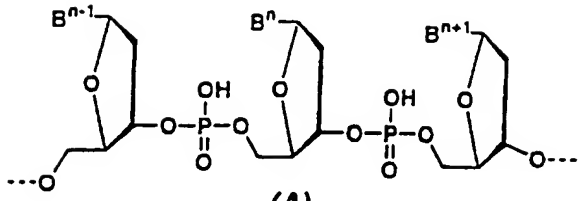
150. A method for the treatment of a disease caused by a pathogenic organism, which comprises administering to a host organism in need of such treatment an effective amount of a pharmaceutical composition according to claim **137**.

151. A method for the treatment of a tumor, which comprises administering to an organism in need of such treatment an effective amount of a pharmaceutical composition according to claim **135**.

152. A method for the treatment of a tumor, which comprises administering to an organism in need of such treatment an effective amount of a pharmaceutical composition according to claim **136**.

153. A method for the treatment of a tumor, which comprises administering to an organism in need of such treatment an effective amount of a pharmaceutical composition according to claim **137**.

Figure 1



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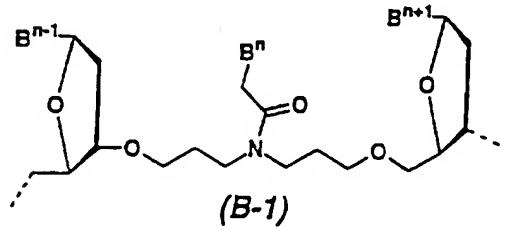

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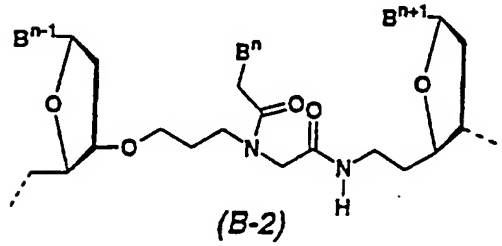

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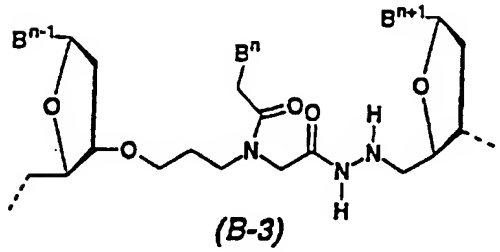

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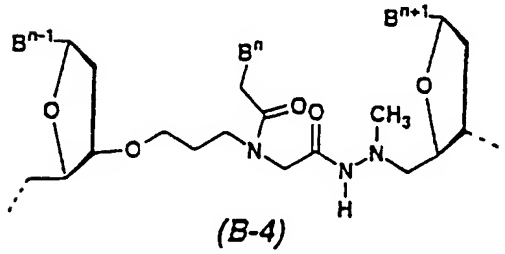

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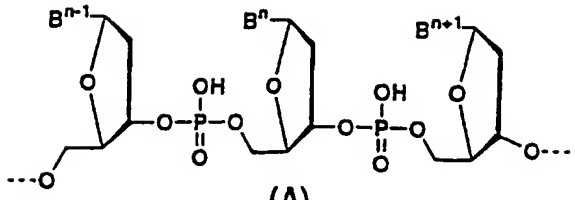

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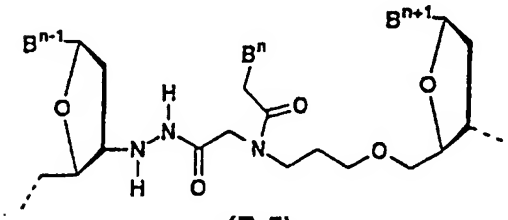

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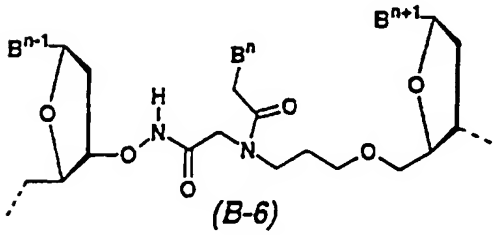

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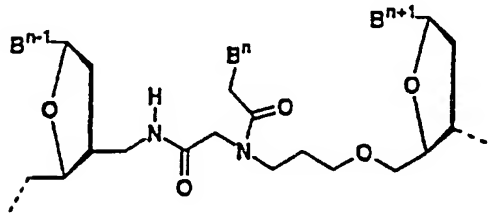

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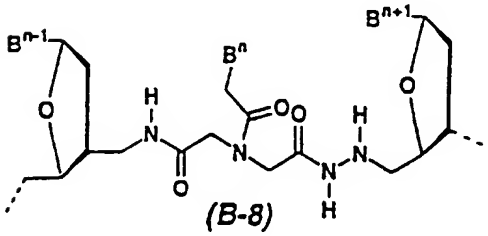

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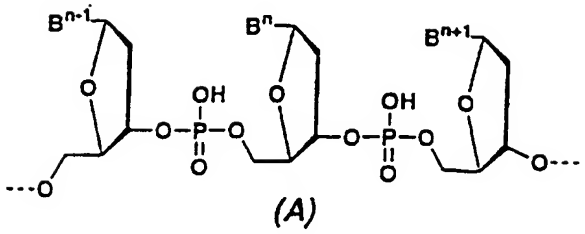

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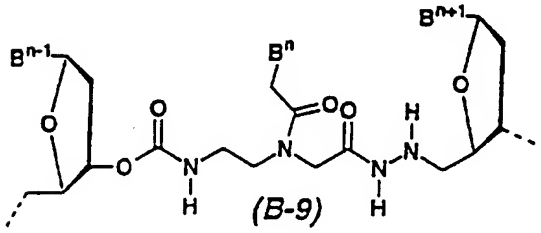

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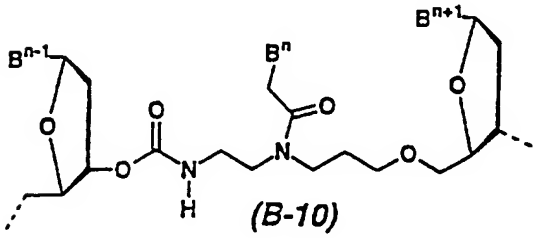

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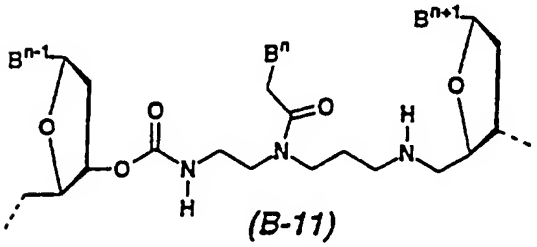

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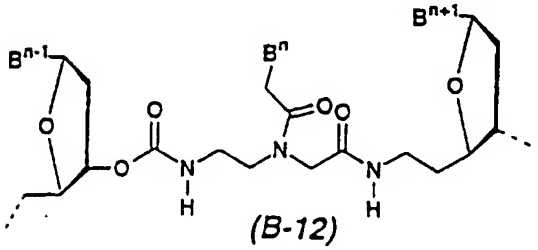

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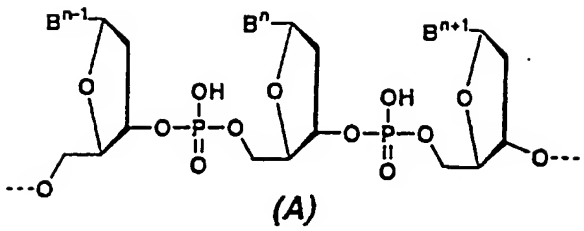

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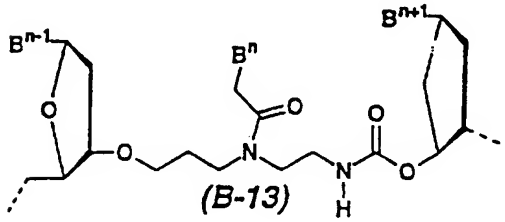

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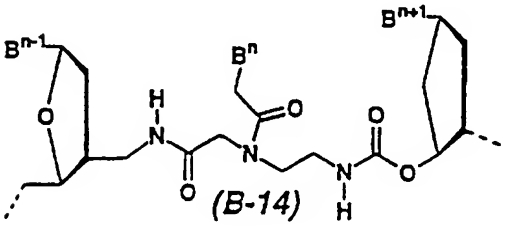

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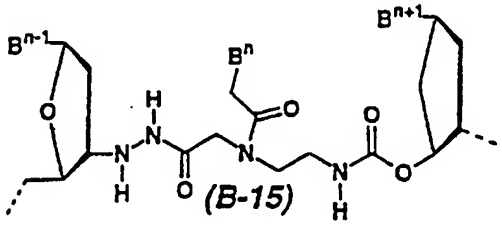

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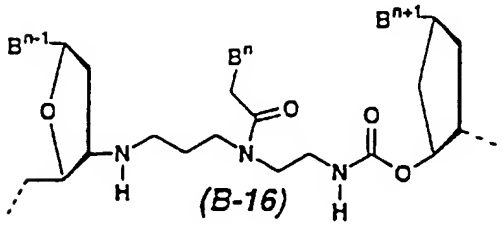

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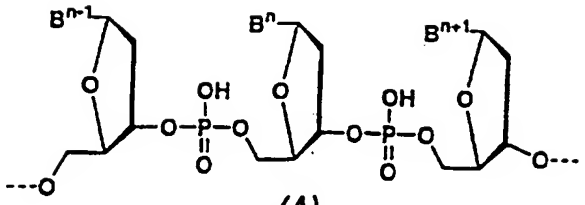

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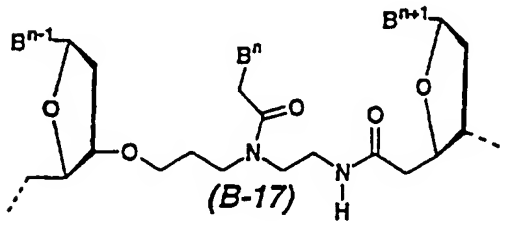

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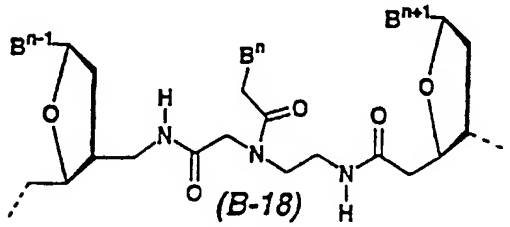

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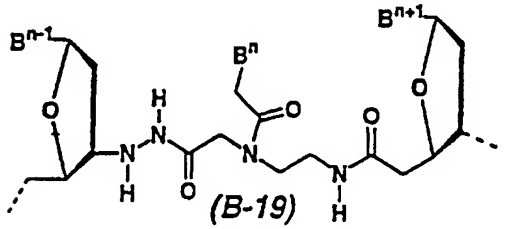

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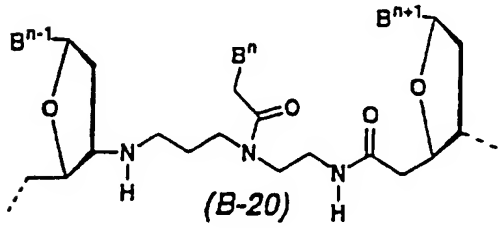

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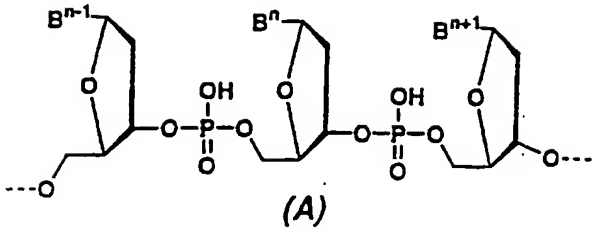

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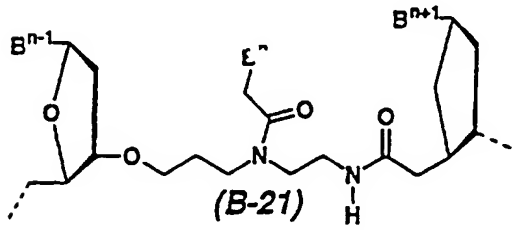

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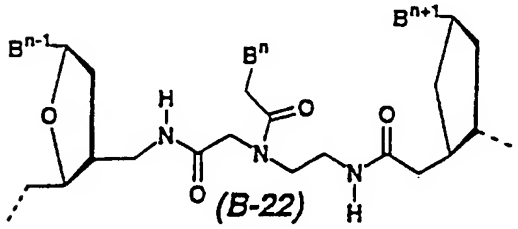

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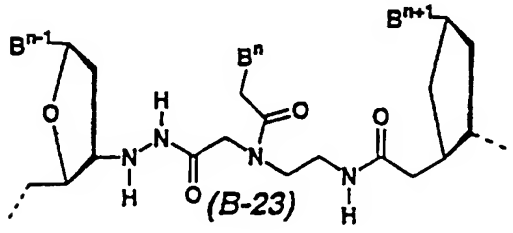

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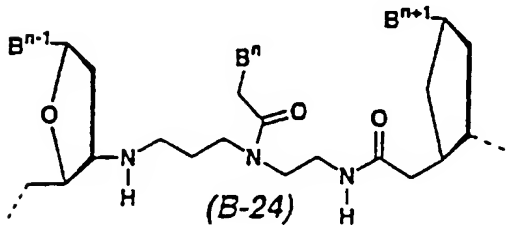

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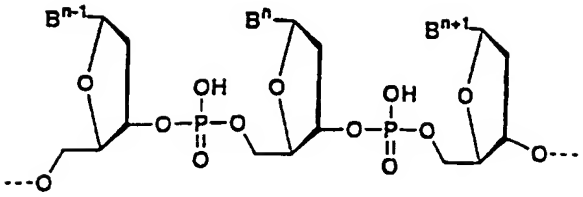

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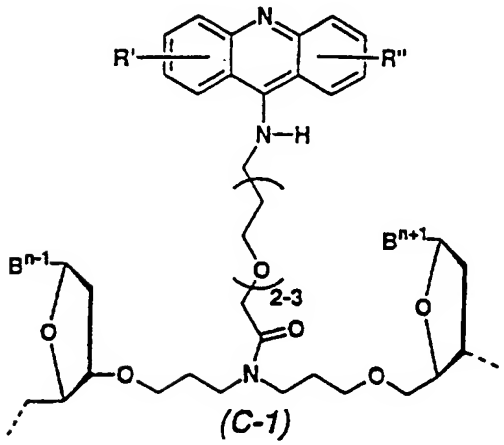

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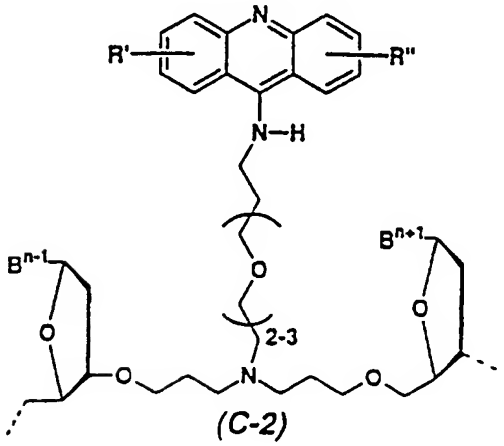

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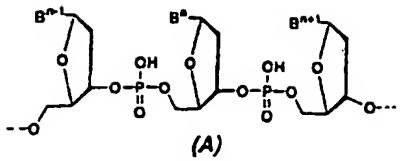

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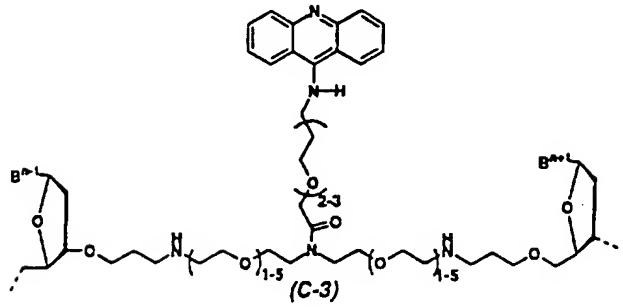

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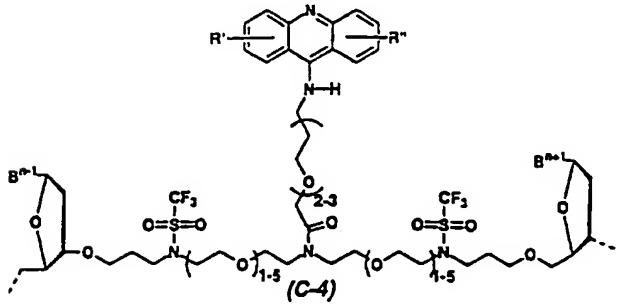

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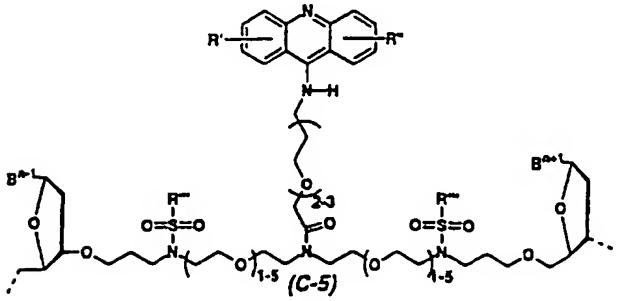

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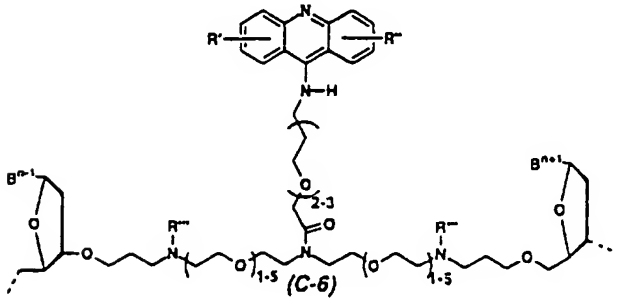

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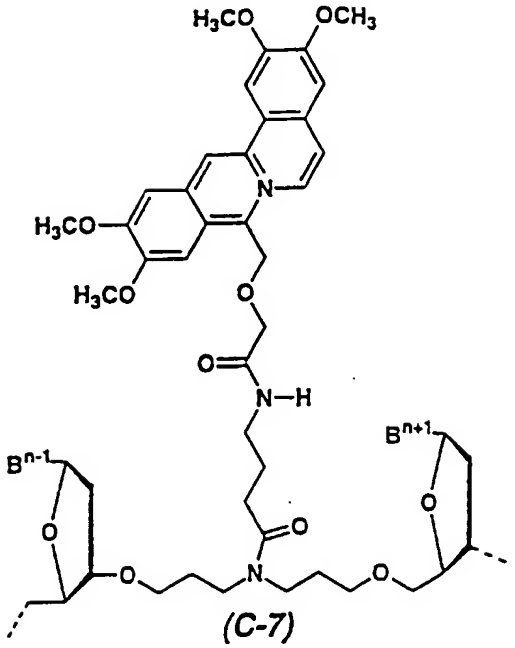

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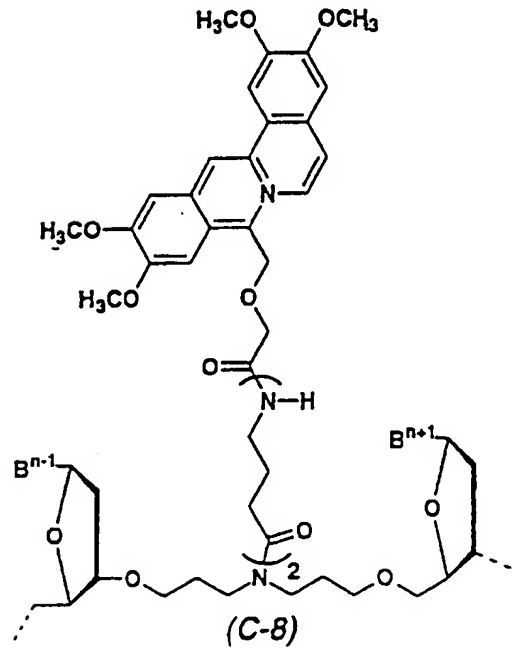

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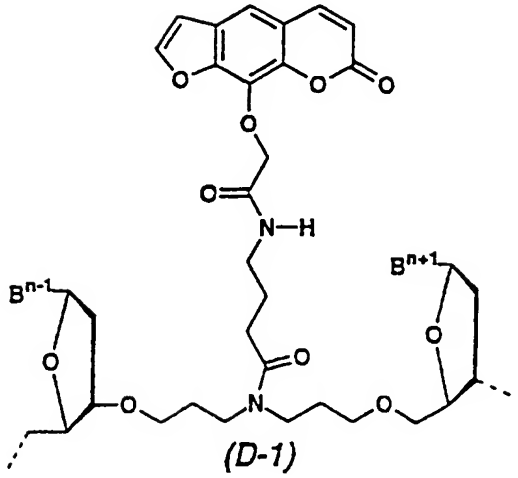

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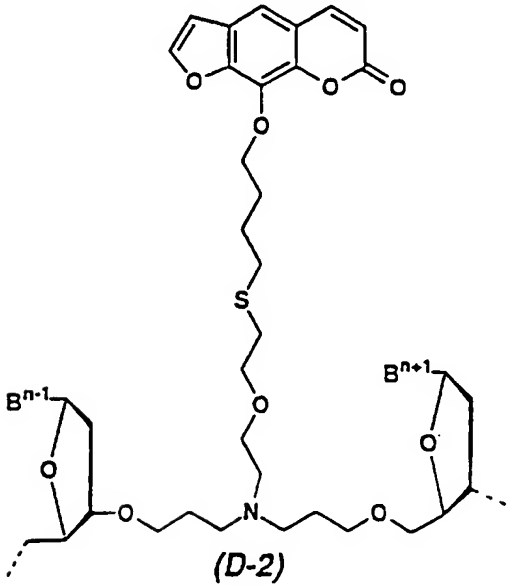

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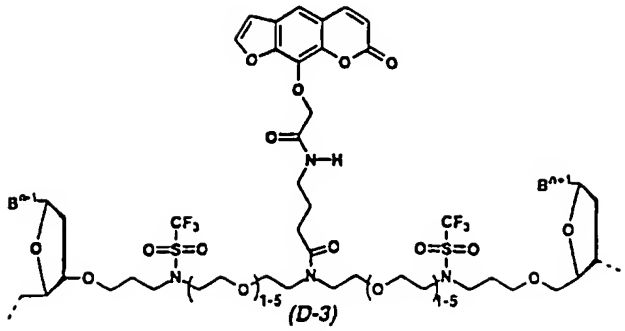

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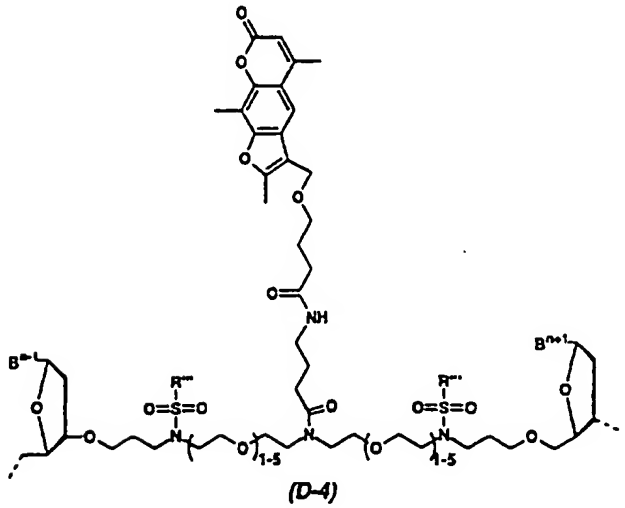

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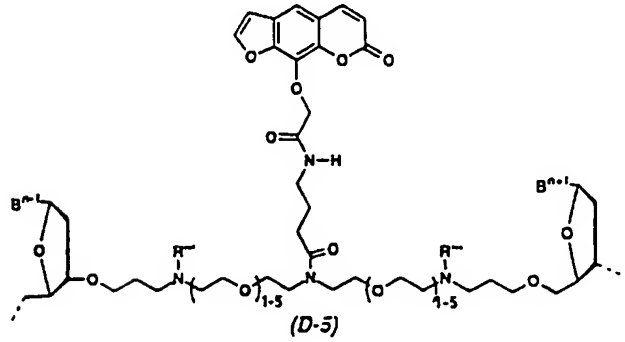

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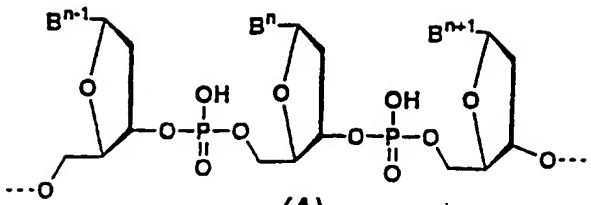

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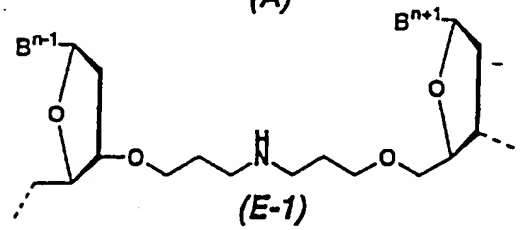

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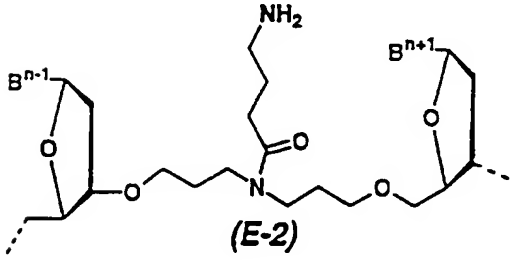

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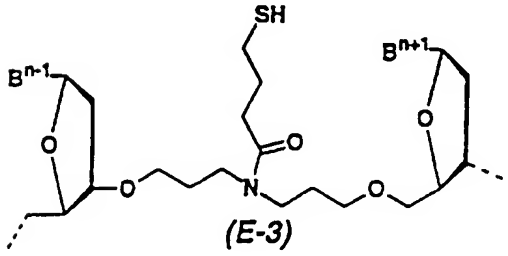
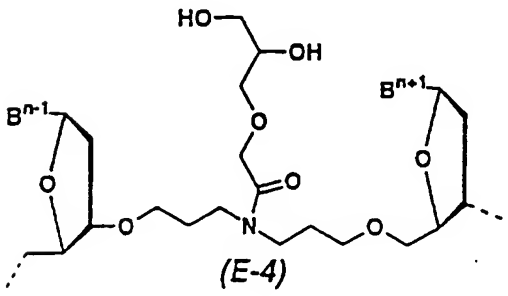
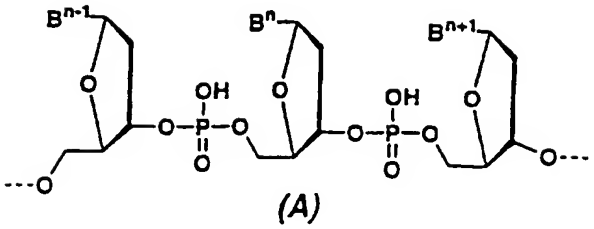


(E-3)

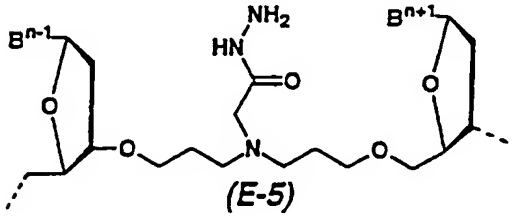


(E-4)

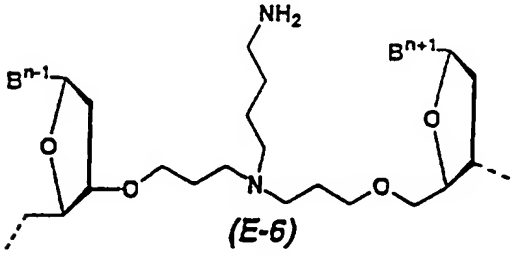
Figure 13



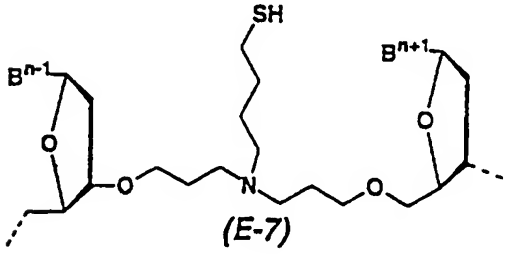
(A)



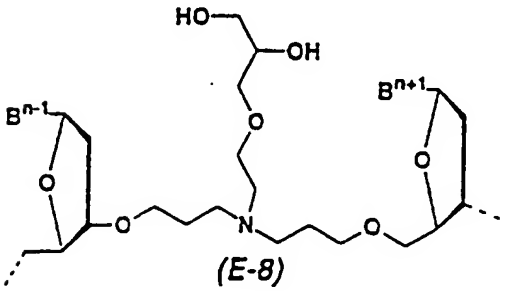
(E-5)



(E-6)

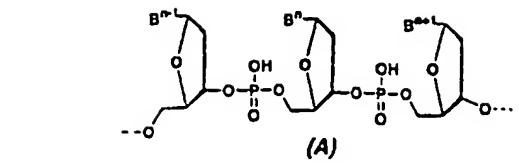


(E-7)

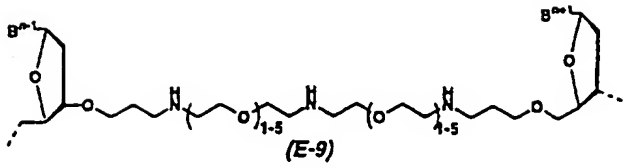


(E-8)

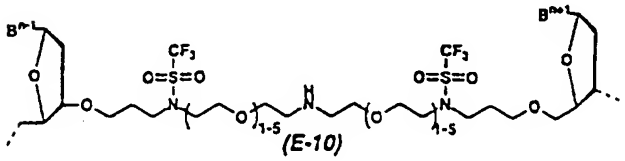
Figure 14



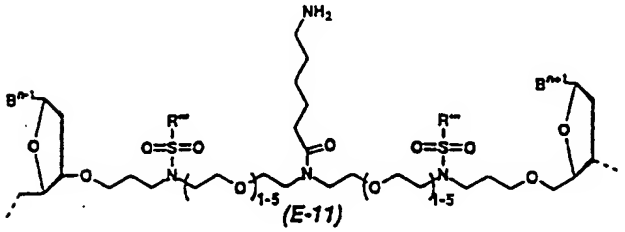
(A)



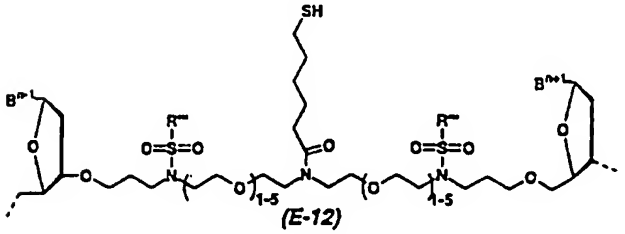
(E-9)



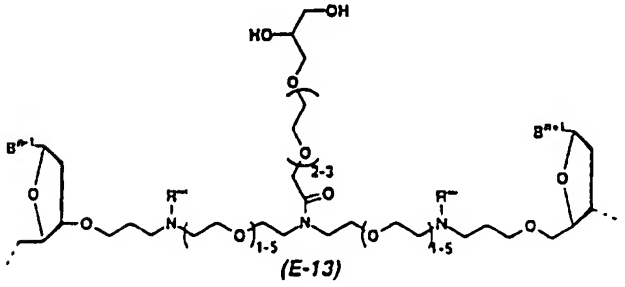
(E-10)



(E-11)

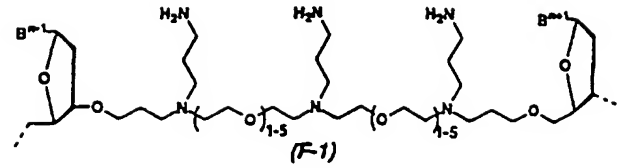


(E-12)

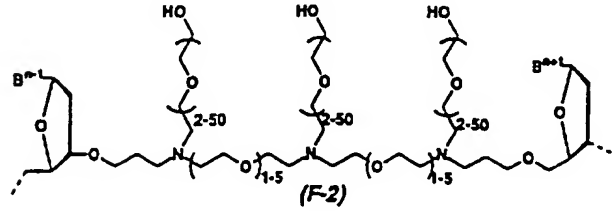


(E-13)

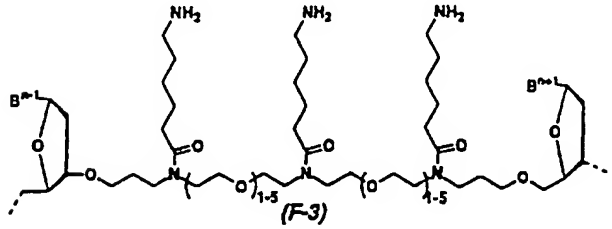
Figure 15



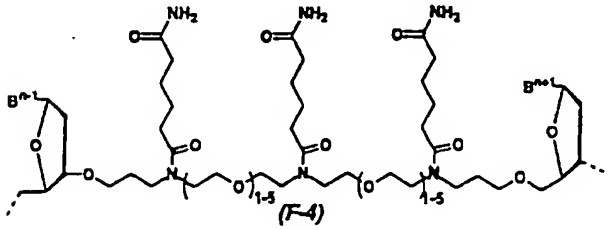
(F-1)



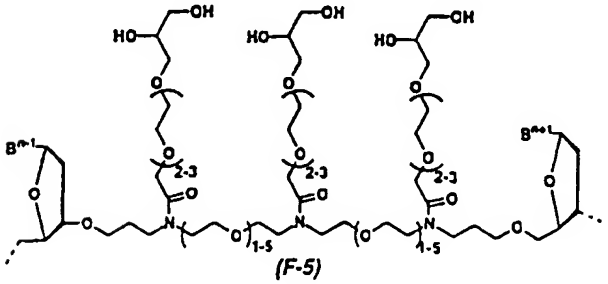
(F-2)



(F-3)



(F-4)



(F-5)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/01236

A. CLASSIFICATION OF SUBJECT MATTER
IPC(6) :C07H 21/00
US CL :536/22.1
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 536/22.1,23.1,24.1,24.3,24.31,24.32,24.33,25.3; 514/44; 435/6; 436/501

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US, 5,386,023 (SANGHVI ET AL.) 31 January 1995, see especially the summary of the invention in columns 3-5 and the in-vivo treatment in columns 57-58.	1,2,5,8,12, 32,33,43,48, 53,56,61,82, 89,96,97,99, 107,111,115, 127,131,135, 142 --- 145,148,151
Y,P	US 5,539,082 (NIELSEN ET AL.) 23 July 1996, see the entire disclosure.	1-153

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search
16 MAY 1997

Date of mailing of the international search report
02 JUN 1997

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231

Facsimile No. (703) 305-3230

Authorized officer
ARDIN MARSCHEL

Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/01236

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 5,393,877 (MCLEAN ET AL.) 28 February 1995, see especially columns 1-8.	1,2,5, 8,12, 32,33,43,48, 53,56,61,82, 89,96,97,99, 107,111,115, 127,131,135 ---------- 142,145,148, 151
Y	US 5,403,709 (AGRAWAL ET AL.) 04 April 1995, see especially column 4, lines 23-34.	1-134
X	US 5,476,925 (LETSINGER ET AL.) 19 December 1995, see especially columns 2-8.	1,2,5,8,12, 32,33,43,48, 53,56,61,82, 89,96,97,99, 107,111,115, 127,131
X --- Y	US 5,124,246 (URDEA ET AL.) 23 June 1992, see especially columns 12-14.	1-15,32-34, 43,48,53,56, 61,82,89, 96-104, 107-109, 111-113, 115-117, 127-129, 131-133 ---------- 135-137, 142- 153

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/01236

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 5,380,833 (URDEA) 10 January 1995, see especially columns 10-12.	1-15,32-34, 43,48,53,56, 61,82,89, 96-104, 107-109, 111-113, 115-117, 127- 129, 131-133 ---------- 135-137, 142-153
X --- Y	JOURNAL OF THE AMERICAN CHEMICAL SOCIETY, Volume 116, Number 7, 1994, "Oligodeoxyribonucleotide N3'->P5' Phosphoramidates: Synthesis and Hybridization Properties", pages 3143-3144, see the entire disclosure.	1,2,5,8,12, 32,33,43,48, 53,56,61,82, 89,96,97,99, 107,111,115, 127,131 ---------- 135,142,145, 148,151

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/01236

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

APS, DIALOG covering CAS, MEDLINE, BIOSIS, EMBASE, WPI, and BIOTECH ABS. over the keywords: oligonucleotid?, oligonucleosid?, phosphoramidit?, link?, nitrogen, antisense, and nuclease resistan?